The occurrence and inheritance of protein variation in plaice, Pleuronectes platessa L.

Thesis

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by

David Thompson B.Sc.
Fisheries Laboratory, Lowestoft.

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ABSTRACT

The occurrence and inheritance of enzyme polymorphism at eight loci in the plaice, Pleuronectes platessa, were examined by starch-gel electrophoresis. The enzymes studied were glycerol-3-phosphate dehydrogenase, malate dehydrogenase phosphoglucomutase, glucose phosphate isomerase A and B, 6-phosphogluconate dehydrogenase, iso-citrate dehydrogenase and adenosine deaminase. Conventional crosses demonstrated the codominant genetic control of polymorphism at these loci. The first five enzymes listed above were used in examinations of broods of induced diploid gynogenetic offspring produced from heterozygous females. The results showed that diploidy was caused by the interruption of meiosis in the developing egg and the retention of the second polar body. Recombination frequencies were used to determine the efficiency of induced diploid gynogenesis in the development of inbred lines; these may be of use in the genetical improvement of commercial fish cultivation. The examination of these same inherited enzyme polymorphisms in induced triploid crosses both confirmed the recombination data and also the triploid nature of the offspring. The production of such triploids, if sterile, could increase the yield of fish farming. Throughout the study the results were examined for evidence of selection and linkage apart from some evidence of selection at the glucose phosphate isomerase-A locus no other examples of either phenomenon were observed. Individual anomalous results, when contamination between broods had been discounted, were interpreted as mutations. The occurrence of polymorphism at the eight loci was examined in samples from different spawning grounds in the North Sea, the Irish Sea and off Iceland. Eggs were collected and returned to the

laboratory for incubation; the resulting larvae were reared to a size suitable for electrophoresis. The results showed that, with respect to these eight loci, the plaice stocks around the United Kingdom appear to be genetically indistinct but differ from those at Iceland at the glycerol-3-phosphate dehydrogenase, phosphoglucomutase and glucose phosphate isomerase-B loci.

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C O N T E N T S

INTRODUCTION

The identification of protein polymorphism by electrophoresis has enabled widespread studies to be made of variation both within and between species. The application of the technique has expanded rapidly in the last two decades with the introduction of stable gel matrices and specific enzyme stains; studies of many genetic loci within a single species have become possible (Hubby and Lewontin, 1966; Lewontin and Hubby, 1966). The occurrence of electrophoretically identifiable polymorphisms in some proteins has been widely reported in natural populations (Powell, 1975) and the role of electrophoretic data in resolving systematic relationships between species has been discussed by Avise (1974). More recently Ayala (1977) reviewed the application of such information in evolutionary studies.

In fish electrophoretic studies have been concerned with both inter and intra specific differences and reviews of the extensive field were made by de Ligny (1969, 1972). Many studies have been concerned with the identification of stocks in species of commercial importance in attempts to assist fisheries management and a special meeting on the subject was held by the International Council for the Exploration of the Sea (ICES, 1971). More recently the application and future use of biochemical polymorphism in fisheries research has been discussed by Jamieson (1974) and by Utter, Hodgins and Allendorf (1974).

Genetical control of polymorphism has been generally assumed either by comparison with family studies made for the same enzymes in other species, usually easily reared laboratory animals or *Drosophila*, or by agreement between the observed and expected

distribution of genotypes computed by the Hardy-Weinberg Principle. Formal genetical studies in fish have been concentrated upon those species with well understood breeding systems for which efficient husbandry techniques exist. Consequently the salmonids, especially those with a history of domestication, have been the subject of much investigation, not only due to their availability and relatively simple husbandry, but also because of the genetical interest caused by their tetraploid ancestry. In commercially important species of marine fish the genetic basis for the interpretation of biochemical polymorphism has seldom been proved by inheritance studies. The aims of this study were to examine the occurrence and inheritance of enzyme polymorphism in the plaice, Pleuronectes platessa, with particular reference to such inheritance in induced gynogenesis and triploidy. Although polymorphisms for the enzymes studied in this project have been described for a range of fish species most accounts have been limited to observations made upon naturally occurring populations.

The plaice, a flatfish of the family Pleuronectidae, order Heterosomata has, because of its economic importance, been the subject of intensive fisheries research, especially in the North Sea, since the end of the nineteenth century (rev. by Wimpenny 1953; Bannister, 1978). The species has a continuous distribution throughout the shallow waters of the continental shelf from the White Sea to the western Mediterranean, with discrete populations at the Faroe Islands and Iceland. It is most abundant within the 40 fathom contour but is found in depths of up to 60 fathoms (Graham, 1956). The life cycle consists of planktonic egg and larval stages followed by metamorphosis and a migration of the juvenile fish to shallow coastal waters. As they become older these

fish gradually disperse into deeper water until in their second or third year they reach maturity and migrate to the spawning grounds.

The first electrophoretic study in plaice was described by Sick, Frydenberg and Nielsen (1963) who examined haemoglobin patterns in the plaice, the flounder, Platichthys flesus, and its naturally-occurring hybrid; interspecific differences were found but no intraspecific polymorphism was observed. However Moller and Naevdal (1967) identified polymorphic forms of transferrin in plaice serum and de Ligny (1967a, b, c) described variations in transferrin allele frequencies between plaice samples from different areas in the North Sea but differences between year-classes and the discovery of an ontogenetic effect (de Ligny, 1970) made interpretation of the data difficult. The same author (1968) published an account of esterase polymorphism in plaice serum but the occurrence of variants was rare, only 10 fish out of a total of 616 tested. Dando (1971) described a similarly low level of polymorphism for lactate dehydrogenase in plaice skeletal muscle, one variant out of 122 fish examined. However, Birley and Beardmore (1972) in a study including 26 enzymes and proteins reported finding polymorphism in 35% of the loci, though it was not until 1977 that a more detailed account of protein variation at 46 loci was published (Ward and Beardmore, 1977). In a different analysis of some of this data the same authors (Beardmore and Ward, 1977) showed that with respect to five polymorphic loci heterozygosity levels in populations of juvenile fish varied with age during their life history. Two loci controlling the formation the enzyme glucose-phosphate isomerase were identified by Dando (1974); each displayed polymorphism and the genetical

interpretation was supported by a small amount of family data.

In another use of electrophoretic data Ward and Galleguillos (1978) applied their knowledge of the amount of protein variation in plaice to compare the evolutionary and systematic relationship between this species, the flounder and the dab, Limanda limanda.

The breeding system of the plaice is very suitable for investigations into the genetic control of protein polymorphism. The production of very large numbers of eggs by each female and their external fertilisation allows large broods of offspring to be reared from individual controlled matings. Indeed by combining the hatchery and rearing techniques developed in fish farming research (Shelbourne, 1964; Riley and Thacker, 1969) with electrophoretic analysis of proteins Purdom, Thompson and Dando (1976) demonstrated the Mendelian control of polymorphism in five enzyme systems, including both glucose phosphate isomerase loci. The 1974 and 1975 data presented in this thesis were included in this paper. Ward and Beardmore (1977) included a small amount of breeding data for four of the loci studied by Purdom et al. (1976); although the numbers of offspring were small they complied with the previously published results.

In this present study the inheritance of polymorphism was examined not only in offspring from controlled normal matings but also in both diploid gynogenetic larvae and induced triploid larvae.

Gynogenesis is a special form of parthenogenesis in which activation of the egg is achieved by fertilization with genetically inert spermatozoa. The process was first described by Hertwig

(1911) in frogs when he fertilized eggs with spermatozoa which had previously been exposed to radium gamma radiation. As the spermatozoa were exposed to increasing levels of radiation the survival rate amongst embryos decreased to zero but then, as the radiation exposure was increased still further, low frequencies of apparently normal embryos appeared. However after further development these embryos revealed severe deformation, consistent with their presumed haploid condition. Indeed Beatty (1964) in a review noted that all the methods of inducing gynogenesis in vertebrates, without exception, produced inviable haploid embryos. To produce viable diploid gynogenetic embryos development of the egg must first be stimulated by inert spermatozoa and then diploidy must be restored by interference either with meiosis or with mitosis.

Whilst discussing the application of breeding systems to marine fish farming Purdom (1969) proposed that induced diploid gynogenesis would offer a rapid means of development of inbred material without the delays associated with several generations of conventional sib-mating. The development of inbred lines followed by their crossing to produce heterosis is an established practice in animal and plant breeding (Lerner and Donald, 1966).

In fish naturally-occurring gynogenesis has been reported in the breeding systems of the Amazon molly, Poecilia formosa (Hubbs and Hubbs, 1932, 1946; Kallman, 1962), the silver crucian carp, Carassius auratus gibelio (Lieder, 1955) and in Poeciliopsis spp. (Miller and Schulz, 1959). In 1961 Romashov, Belyaeva, Golovinskaia and Prokof'eva-Bel'govskaya successfully produced diploid gynogenomes in loach, Misgurnus fossilis, carp, Cyprinus

carpio, and sturgeon, Acipenser ruthenus, from eggs fertilized with irradiated spermatozoa and then subjected to a cold shock treatment. Purdom (1969) using a similar technique produced gynogenetic diploid offspring in plaice. In a review of induced diploid gynogenesis in fish Stanley and Sneed (1974) list 17 species in which successful manipulation of the embryo has been achieved.

If diploid gynogenesis is to be used as a technique for rapid inbreeding the method by which diploidy is induced is important. Purdom (1969) quoted Tyler (1941) and Beatty (1964) in suggesting three methods of restoring diploidy after gynogenetic activation of the egg:

i. the retention of a product of the polar body from the first division of meiosis and its fusion with the egg pronucleus;

ii. the retention of the polar body from the second division of meiosis and its fusion with the egg pronucleus;

iii. the suppression of cleavage following the first mitotic division of the zygote.

The first method would be equivalent to self-fertilisation, in the second the degree of inbreeding would depend upon the frequency of crossing-over per chromosome in the first meiotic division and the third method would result in total homozygosity.

Although the third method is reported to operate in frogs (Parmenter, 1933; Kawamura, 1939; Subtelny, 1958) the lack of cleavage delay in gynogenetic diploid plaice embryos and the

absence of diploid androgenesis (Purdom, 1969) suggests that in fish this is not the explanation for the re-establishment of diploidy. Of the remaining two possibilities the first is unlikely:the first polar body would have to undergo a meiotic division and be partly retained and also teleost eggs at ovulation are already undergoing the second meiotic division (Ginzburg, 1968).

The application of electrophoretic techniques specifically identifying genetically controlled polymorphic enzymes can provide precise data as to which method occurs in plaice. The segregation into different genotype classes of gynogenetic offspring from females displaying heterozygosity for a particular enzyme system should identify the method of diploidisation. If the first method applies at least half of all the offspring should be heterozygous, similar to the parent ; in the second method both types of homozygote and the heterozygote should be found, when the proportion of heterozygous offspring produced reflects the frequency of crossing-over between the locus and the centromere; finally the third method would result in the offspring segregating into both classes of homozygote. If the first and third methods do not apply cross-over frequencies become important in estimating the efficiency of induced diploid gynogenesis; a major aim of this project was to determine these frequencies at as many loci as possible within the practical limitations of rearing and testing large numbers of juvenile plaice.

Following the successful production of gynogenetic diploid plaice, Purdom (1972) reported the production of triploids in plaice and in the hybrid between plaice and flounder,

a cold shock treatment was used, as in gynogenesis, but this time following normal fertilization. Both natural and induced triploidy had previously been reported in amphibia (Fankhauser, 1938, 1945) but in fish, apart from plaice-flounder hybrids, triploid adults have only been reared in sticklebacks, Gasterosteus aculeatis (Swarup, 1959) and in hybrids between Poecilia species (Rasch, Darnell, Kallman and Abramoff, 1965). Naturally-occurring triploids have been found in cyprinids (Cherfas, 1966) and in Poeciliopsis species (Schulz, 1967).

Once again the electrophoretic analysis of broods of offspring from parents of appropriate genotypes can provide information as to the method of triploidisation and substantiate any crossover data obtained from diploid gynogenesis. Triploidy may be of further importance in fish farming. In normally diploid species induced triploids should be sterile, since the gametic meiosis will be abnormal. Consequently gonad development may be reduced and food conversion, growth and other physiological factors of economic significance in fish cultivation may be affected. Purdom (1972) used morphological and histological characters to identify triploids; the use of inherited enzyme polymorphisms may be more informative.

As previously stated the genetical control of biochemical markers used in the identification of marine fish stocks has seldom been demonstrated by family data. Therefore as a demonstration of the potential of hatchery techniques and the genetical identification of fish stocks juvenile plaice, reared in the laboratory from eggs collected from different spawning areas, were typed for the loci

examined in the inheritance studies. The data obtained were examined for any genetical differences between areas.

The major aims of the project were as follows:

i. to examine the mode of inheritance of as many polymorphic enzyme systems as possible; within the limitations of sampling live adults and rearing, maintaining and testing many broods of larval offspring;

ii. to determine the method of diploidisation by the segregation of alleles in gynogenetic diploid offspring derived from females heterozygous for one or more of the loci tested above and to use any observed recombination data to estimate cross-over frequencies between each locus and the centromere in order to assess the relevance of gynogenesis in fish cultivation;

iii. to repeat these experiments where possible in induced triploid offspring from parents of selected genotypes;

iv. to combine the hatchery techniques developed at Lowestoft with the use of biochemical markers under known genetical control to survey spawning aggregations of plaice.

v. to present information on linkage and mutation rates obtained as a result of these studies.

MATERIALS AND METHODS

A i COLLECTION OF ADULT PLAICE

For the experiments described in this study it was necessary to collect ripe male plaice and female plaice due to spawn within 7 to 14 days for return alive to the laboratory. To do this adult plaice were caught each year by trawling from either the MAFF research vessels CLIONE and CORELLA or, for the 1978 experiments, from the inshore fishing vessel TWO BROTHERS of Folkestone. Table 1 gives details of these collections.

For the 1974, 1975 and 1976 experiments fish were taken from various known spawning grounds in the English Channel and North Sea. The dates of capture depended upon the availability of research vessels and the location upon the state of sexual maturity of the fish on the different spawning grounds (Simpson, 1959). In preparation for the 1977 experiments some immature males were collected in September 1976 and kept in the laboratory until sexual maturity was reached in the spring of 1977. The remainder of the fish for 1977 were collected in the usual way.

Throughout each of these four years, despite careful selection on board ship, considerable mortality was experienced once the fish had been returned to the laboratory. This mortality was due to the method of capture, although in 1977 some losses occurred because of a failure in the laboratory's sea water supply. Fishing with a heavy bottom trawl from a large vessel caused considerable physical damage to the fish. Females which were almost ready to spawn were particularly susceptible to damage; the smaller males were less affected.

TABLE 1 Details of collection of live mature plaice 1974-78

Date of capture	Locality (fishing ground)	Position		Number of fish	
		Lat N	Long E	♀	♂
4 & 5/1/74	Tea Kettle	52°23'N	03°27'E	17	7
6/1/74	Flamborough Off	53°58'N	01°05'E	5	4
7/1/74	Silver Pits	54°08'N	02°45'E	17	8
17/1/74	Kentish Knock	52°10'N	02°10'E	6	-
1 & 2/2/75	West Mud Hole	53°40'N	03°20'E	33*	11
15 & 16/12/75	Dover Strait	51°30'N	01°45'E	6	1
14/1/76	Silver Pits	54°08'N	02°20'E	10	6
25 & 26/1/76	West Mud Hole	53°40'N	03°20'E	34	27
21/9/76	Sole Bay	52°15'N	01°45'E	-	9
4 - 9/2/77	West Mud Hole White Bank Silver Pits	53°45'N 54°55'N 54°10'N	03°10'E 06°03'E 02°33'E	))62)**	26
2 & 3/11/77	Dover harbour	51°08'N	01°20'E	24	10
15/1/78	Smiths Knoll	52°50'N	02°20'E	10	4
6-10/2/78	Eastern Deeps Brown Ridges	52°05'N 52°40'N	03°10'E 03°15'E	)7)	8

*5 ♀ and 1 ♂ plaice retained for 1976 season

**2 ♀ and 4 ♂ plaice retained for 1978 season

To avoid this heavy loss of fish a small inshore fishing boat, using a small light bottom trawl, was hired for the 1978 season. The fish caught by this vessel were in excellent condition and survived the whole season. Some fish were also collected as previously from MAFF vessels.

In all years the fish were returned to the Lowestoft laboratory and kept outside in a large tank (4 m x 4 m x 1 m), with a throughflow of sea water (5 l/minute), aeration and biological filtration were used to prevent build up of waste products.

At the laboratory the fish were tagged either with numbered Petersen discs or by cold branding. The latter method was more frequently used as individual fish could easily be recognised at a distance. Fish were numbered by marking a dried area of skin with a cold brass branding iron. These irons were cooled in a mixture of absolute alcohol and solid carbon dioxide. Males were branded below the lateral line; i.e. on the gonad, females above, i.e. away from the gonad.

At the same time tissue samples were taken. A small amount of white skeletal muscle was removed from each fish either by biopsy needle or by lifting a small flap of skin just before the caudal fin and excising a small piece of tissue with a scalpel. Incisions were immediately treated with antibiotics to aid healing and prevent infection. The tissue samples were kept in sealed polythene bags ready for electrophoresis and were always tested within a few hours of collection (see materials and methods, B: electrophoresis).

Normally these fish were kept only for a few weeks until sexually spent. However in 1975 and 1977 small numbers of fish were retained for use in 1976 and 1978 respectively. This was not a routine practice due to lack of facilities and the uncertainty of spawning of captive fish.

Spawning fish were not fed but at other times of the year fish were fed on alternate days with live lugworms (Arenicola marina) at the rate of 3 or 4 worms per fish.

A ii COLLECTION OF EGGS AND JUVENILES FOR POPULATION STUDIES

In 1975 live plaice eggs were collected by research vessels from three known spawning areas in the North Sea and returned to the Lowestoft laboratory for incubation and larval rearing. In September 1976 samples of '0' group juvenile plaice i.e. fish spawned earlier in the same year, were taken from three beaches on the east coast of England. In 1977 the geographical range of egg collection was extended to include the Irish Sea, more areas of the North Sea and the Moray Firth. In 1978 eggs were obtained from Icelandic waters. Tables 2A and B and Figure 1 give details.

Although it is not known whether plaice eggs are released and fertilized near the sea bed or in midwater (Harden Jones, 1968), viable eggs rise to the surface and are easily collected by towing a 2 m diameter ring net gently through the upper layers of the sea. The eggs were returned to the laboratory either in plastic bins or 4 l thermos flasks depending on distance and method of transport. Incubation and rearing will be described later.

Juvenile plaice were caught by a 1 m push net at a half to one metre depth at low water. These fish were immediately placed

TABLE 2A Details of collection of plaice eggs

Sample number	Date of collection	Locality	Position
1	30/1/75	Gabbard	51°53'N 02°20'E
2	31/1/75	West Mud Hole	53°43'N 03°27'E
3	9/3/75	Castle Ground (Off Flamborough)	54°30 N 00°15'E
4	26/1/77	Gabbard	52°05'N 02°22'E
5	7/2/77	West Mud Hole	53°40'N 03°14'E
6	8/2/77	White Bank	54°56'N 05°56'E
7	9/2/77	NE Cleaver Bank	54°26'N 03°40'E
8	21-23/2/77	Moray Firth	58°10'N 03°15'W
9	4/3/77	Off Maughold Head (Isle of Man)	54°19'N 04°12'W
10	12/3/77	Scarborough Ground (Off Flamborough	54°20'N 00°21'E
11	17/4/78	Off Reykjanes (SW Iceland)	63°55'N 22°45'W
12	1/5/78	Off Reykjanes (SW Iceland)	63°55'N 22°45'W

TABLE 2B Details of collection of '0' group plaice

Sample number	Date of collection	Locality	Position
13	4/10/76	Lowestoft Beach	52°28'N 01°45'E
14	14/10/76	Cleethorpes Beach	53°32'N 00°02'W
15	20/10/76	Filey Beach	54°10'N 00°15'W

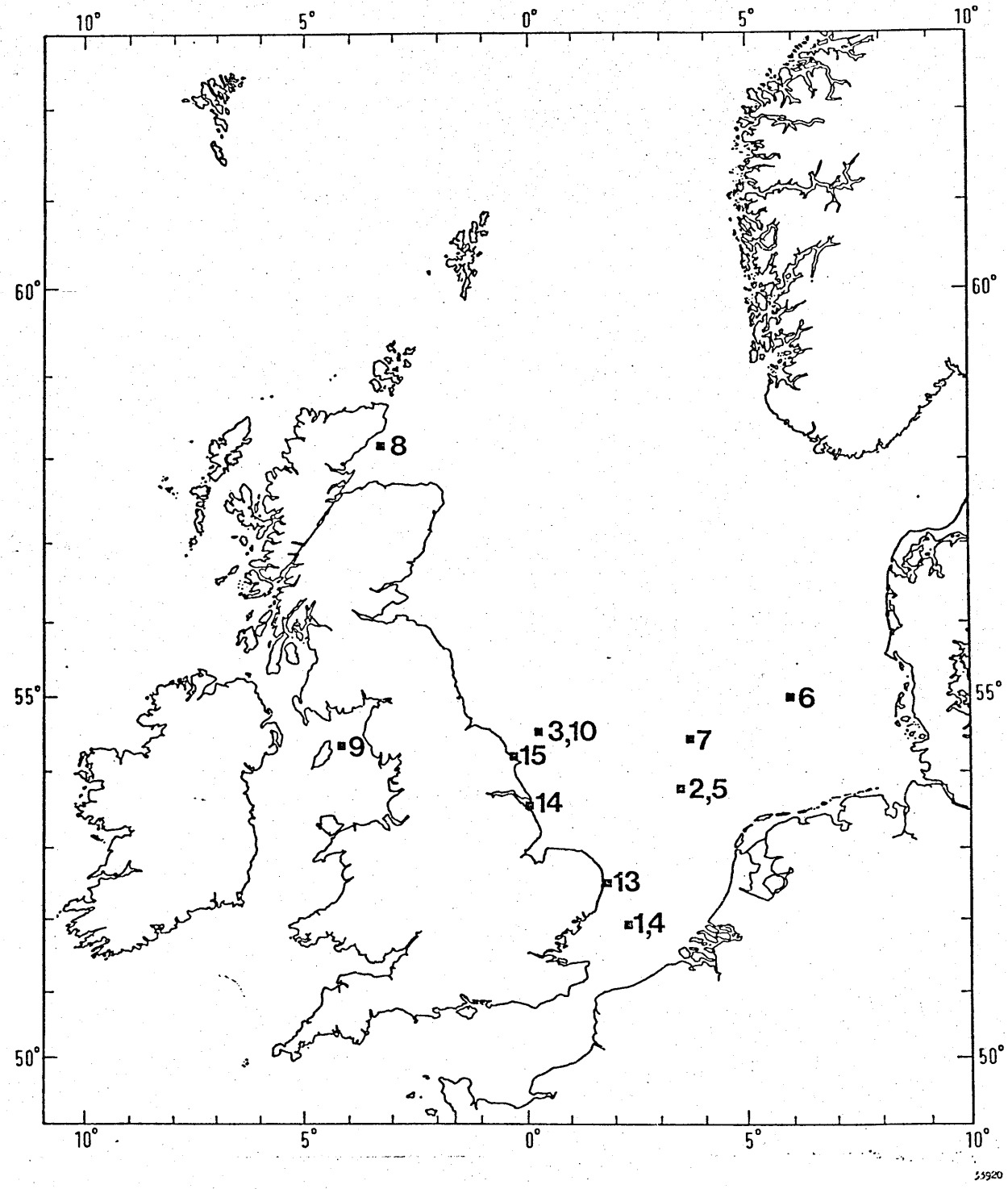

Figure 1 : The positions are shown of the egg and juvenile plaice samples collected in the North and Irish Seas. The numbers correspond to those used in Table 2. Samples 11 and 12 were collected five miles off the coast of South West Iceland : a position 1200 miles from Lowestoft.

in individual polythene bags and frozen at -20°C. These fish were between 3 and 5 cm long and were products of the 1976 spawning.

A iii CROSSES, COLD SHOCKING AND INCUBATION

Three types of crosses were carried out to produce either normal diploid offspring, gynogenetic diploid offspring or induced triploid offspring.

Controlled matings were made between fish which were heterozygous or of unlike homozygosity for any one or more of the enzymes tested. Eggs were hand stripped from mature females into 2 l glass crystallizing bowls containing 250-300 ml sea water. Milt was stripped from males by hand pressure and was pipetted into the bowls containing the eggs. Care was taken to ensure that the correct crosses were made and that no contamination took place from one bowl to another. The eggs and milt were swirled round to aid fertilization. After twenty minutes the bowl was topped up with 1.5 l of clean sea water and the eggs were incubated at 7°C. Any inviable eggs soon sank to the bottom and were removed by pipette. Thereafter the water was changed each day, accompanied by the removal of any dead eggs. A constant incubation temperature was maintained by keeping the clean sea water and the bowls of eggs in the same room. Hatching began after 10 days. This technique has been used extensively for different flatfish species at Lowestoft and was first described by Riley and Thacker (1969).

A different method of incubation was used in 1978 when eggs were incubated in cylindrical bags, 14 cm in diameter and 22 cm deep, made from monofilament nylon mesh, 13 mesh per cm and

suspended in a tank (60 cm x 135 cm x 60 cm) of sea water at 7°C. This tank had a biological gravel filter which ensured a circulation of aerated clean water through the mesh bags. Dead eggs still had to be removed from the bottom of the bags but the disturbance of eggs caused by water changes in the previous method was avoided. Of equal importance the quality of water in the tanks remained uniformly high whereas in the bowls this gradually deteriorated between water changes.

Gynogenetic diploid plaice larvae were produced by fertilizing plaice eggs, from a female heterozygous at one or more of the loci studied, with either milt from a male Atlantic halibut (Hippoglossus hippoglossus) or with irradiated plaice milt. Fertilization was followed by a cold shock treatment. The use of irradiated plaice milt in producing gynogenetic diploid larvae was first described by Purdom (1969). Its replacement by halibut spermatozoa to produce 'false hybrids' was reported by Purdom and Lincoln (1974).

In 1975, 1976 and 1977 milt from male halibut kept in laboratory tanks was used. These fish had been collected by trawling on the Faroe Bank in May 1971. Milt was obtained in a similar manner to plaice except that greater quantities were collected from each individual. It was found that milt could be stored for up to 5 days in a refrigerator at 4°C without loss of potency.

The method of artificial fertilization was as previously described for the normal matings except that cooled sea water at -1.7°C was used to top up the bowls after fertilization. This produced a final temperature of approximately 0°C which was

maintained by either placing the bowls in a constant temperature room at $0^{\circ}C$ or in an insulated container containing a crushed ice/sea water mixture.

After 3 hours the bowls were removed to a $7^{\circ}C$ environment and allowed to equilibrate. The timing and duration of the cold shock treatment was determined by Purdom (1969).

In 1978 irradiated plaice milt was used as halibut were no longer kept at Lowestoft. Milt samples were held in sealed glass tubes on ice and irradiated with ^{60}Co gamma rays at a dose of 100,000 rad delivered in 257 minutes. This is the dosage required to completely de-activate the genetic material of the spermatozoa (Purdom, 1969). Irradiated milt found to remain viable for up to 4 days when stored in a refrigerator at $4^{\circ}C$.

Incubation of the eggs was carried out according to one or other of the methods already described.

Induced triploidy in plaice was first described by Purdom (1972). The associations of artificial fertilization and cold shocking were used as previously described to produce gynogenetic diploid larvae except that normal plaice spermatozoa were used. In these crosses heterozygous females were always used and, where possible, males were selected which were homozygous for a third allele not present in the female. Egg incubation was as previously described.

The eggs collected at sea from spawning grounds in 1975, 1977 and 1978 were also incubated by one or other of the previously described methods, depending upon the year. The plaice eggs were usually easily identified from any other fish eggs in the sample by size (Russell, 1976). Any unresolved identification

was decided after hatching by larval difference (Nichols, 1971).

A iv LARVAL REARING

After hatching larvae were either tested within a few days or transferred to black polythene tanks (46 x 26 x 15 cm deep) containing approximately 10 l of either settled or filtered sea water. Whether or not larvae were immediately tested depended upon the enzyme to be studied (see materials and methods, B: electrophoresis). For immediate testing the larvae were left in either the 2 l glass bowls or the nylon mesh bags and picked out when required for electrophoresis. However, for further rearing 200 larvae were placed in each black tank. At 7°C plaice larvae take 7 days to absorb the yolk sac. At this time the tanks were moved to an 11°C environment and the larvae were fed daily with newly hatched nauplii of the brine shrimp Artemia salina. The tanks were cleaned of faeces and dead Artemia and larvae daily. In 1974, 1975 and 1976 the larvae were reared under static conditions but in 1977 and 1978 aeration was provided in an attempt to improve both survival rates and water quality. Metamorphosis was completed after 6-8 weeks and just after this, when the fish had reached a length of about 15 mm, they were killed. The fish were either tested electrophoretically immediately or stored individually in polythene bags at -20°C. In some cases rearing was continued for several months. In these instances the fish were fed on the white worm Euchytraeus albidus. In no experiments were fish reared for longer than 6 months.

B. ELECTROPHORESIS

The use of an electrical current to cause the migration and separation of serum proteins through a liquid medium was first described by Tiselius (1937) and the theory of electrophoresis has been described by Bier (1967). The history of electrophoresis has been reviewed by Brewer (1970). The most significant advances for research purposes were the introduction of starch as a matrix for gel electrophoresis (Smithies, 1955) and the development of histochemical staining of gels (Hunter and Markert, 1957). Comprehensive accounts of the methods of gel preparation, the choice of buffers and the techniques of staining gels for specific enzymes have been published by Smith (1968), Brewer (1970), Shaw and Prasad (1970) and Harris and Hopkinson (1976).

Starch gel electrophoresis, in both horizontal and vertical forms, as first described by Smithies (1955 and 1959, respectively) was used throughout this work. Gels were made with hydrolysed starch (Connaught Medical Laboratories) in concentrations of either 12 or 15 per cent weight/volume depending upon the buffer system. Table 3 gives details of constituents and electrophoretic conditions. All the gels were prepared by adding the buffer to the starch in a round bottomed boiling flask and heating the mixture in an electric mantle. The mixture was constantly stirred by an electrically driven glass paddle clamped above the mantle and flask. Just before the mixture boiled and after passing through the viscous stage, the flask was taken from the mantle and all the air bubbles removed from the hot starch by boiling under a reduced pressure from a vacuum pump. The mixture was then poured into a

Table 3 Details of electrophoresis

Method	Starch concn.	Buffers	Reference	Rate and time of electrophoresis
1. Vertical	12%	Stock 0.9 M Tris (hydroxymethyl) methylamine 0.5 M Boric acid 0.002 M Ethylene diaminetetracetric acid disodium salt pH 8.7 Gel: 1:20 dilution Bridge: Upper chamber 1:7 dilution Lower chamber 1:20 dilution	Boyer, Fainer and Naughton (1963) wi th modification by Dando (1970)	200 V across length of gel for 17 h
2. Horizontal	12%	Gel 0.002 M Citric acid adjusted to pH 6.9 with N-(3-aminopropyl) diethanolamine Bridge 0.04 M Citric acid adjusted to pH 6.9 with N-(3-aminopropyl) diethanolamine	Clayton and Tretiak (1972)	4 V/cm of gel for 17 h
3. Horizontal	15%	Gel 0.005 M L. Histidine monohydrochloride adjusted to pH 7.0 with 0.1 M Sodium hydroxide Bridge 0.41 M tri-sodium citrate adjust to pH 70 with 0.5 M citric acid	Brewer (1970)	9 V/cm of gel for 4 h
4. (a) Vertical (b) Horizontal	12%	Gel 0.076 M Tris (hydroxymethyl) methylamine 0.005 M Citric acid pH 8.65 Bridge 0.3 M Boric acid 0.05 M Sodium hydroxide pH 8.2	Poulik (1957)	(a) 100 V across length of gel for 17 h (b) 4 V/cm of gel for 4 h
5. Horizontal	12%	Gel 0.013 M Tris (hydroxymethyl) methylamine 0.004 M Citric acid pH 7.0 Bridge 0.38 M Tris (hydroxymethyl) methylamine 0.14 M Citric acid pH 7.0	Syner and Goodman (1966)	4 V/cm of gel for 17 h

mould.

Gels for horizontal electrophoresis were made in perspex frames with internal dimensions 152 mm x 183 mm x 6 mm (Shandon Scientific Company Ltd.) held onto 3 mm thick glass plates by rubber bands. 250 ml of buffer was used for each gel.

Vertical gels were made with 650 ml buffer per gel and were poured into perspex moulds with internal dimensions 317 mm x 171 mm x 6 mm. At each end of the gel the thickness was increased gradually over the last 35 mm to a final thickness of 28 mm to give better contact between the gel and buffer chambers. Removable plates 184 mm x 34 mm were screwed across these ends to complete the mould until the gel had cooled and set. Silicon grease was used to seal the joints between these removable pieces and the main part of the mould. 6 mm thick perspex was used throughout.

The gels were allowed to cool at room temperature until they had set sufficiently to allow their removal to a refrigerator at 4°C for 30 minutes.

Uniform thickness was ensured by slicing off the meniscus formed by excess gel with 36 swg stainless steel suture wire stretched between two pairs of artery forceps.

Throughout the experiments samples were either white skeletal muscle or whole larvae. It was not necessary to homogenise either the small amounts of tissue taken from the parent plaice or the very small larvae which were used. In experiments where larvae were reared beyond metamorphosis small amounts of tissue were taken posterior to the vent. Quite satisfactory resolution of electropherograms was obtained by placing small

amounts of muscle or whole larvae straight into the gels. Using a microspatula, small 4 mm wide slits were made in the gels across a line 60 mm in from one end. As each slit was formed, a sample was placed into it using pointed watchmakers forceps. Experiments were carried out using removable slot formers placed into the gels before setting, but these formers were found to be an unnecessary sophistication and introduced the problems of sealing slots and maintaining isolation from one sample to another.

After insertion of the samples the gels were covered with thin polythene sheeting. Horizontal gels were placed across two plastic containers, 190 mm x 110 mm x 70 mm (Stewart Plastics Ltd) each containing 250 ml of bridge buffer. Contact was made between the buffer and the gel by a folded length of absorbent surgical gauze. Stainless steel wire electrodes were placed in each tank and connected to a stabilized power supply (either Baird and Tatlock (London) Ltd or Roband Electronics Ltd). The correct voltage per cm of gel was achieved by measuring the voltage over 10 cm of gel and adjusting the output from the power supply accordingly. After the removal of the end pieces vertical gels were fastened by elastic bands to a perspex stand (see Figure 2). The gel stood immersed in the lower buffer chamber, upon a folded piece of surgical gauze and was connected to the upper buffer chamber, again by surgical gauze. Electrodes, connections and power supplies were similar to the horizontal gels. The voltage between the top and the bottom of the gel was measured and the power supply adjusted as before to produce the required reading. All gels were run in a refrigerator

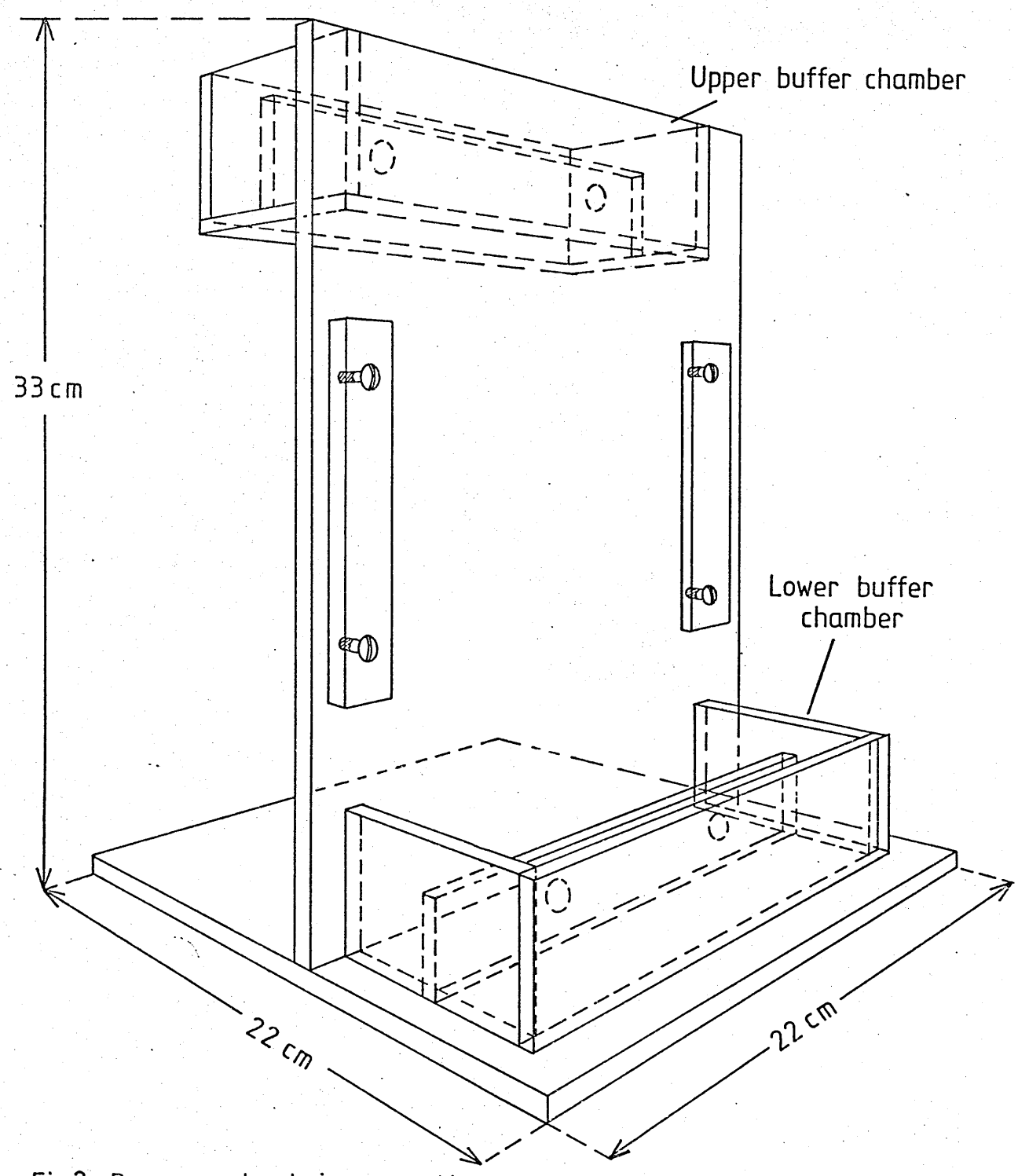

Fig 2. Perspex stand, incorporating upper and lower chambers used for vertical starch gel electrophoresis.

at 4°C; leads from power supplies were thin enough not to break the airtight rubber seal around the door. No other cooling such as water jackets or ice bags around gels, was found to be necessary.

After electrophoresis the gels were removed from the moulds and cut into either two or three slices depending upon the number of enzymes under observation. Suture wire, as previously described, was used to cut the gels and perspex strips down the gel sides gave the required slice thickness.

All the gels were stained by the agar gel overlay technique. Details of enzymes tested and by which electrophoretic method, are given in Table 4. Two per cent weight/volume of agar (Oxoid Ltd, London) in distilled water was used throughout. The agar was boiled and then allowed to cool to about 60°C before use. The stain constituents were dissolved in their respective buffers and an equal volume of hot agar gel added. After stirring the warm mixture was poured onto the cut surface of a slice of gel. Where enzymes in suspension were used as part of the stain, i.e. glucose-6-phosphate dehydrogenase, xanthine oxidase and nucleoside phosphorylase, these were added last, just before pouring the stain over the gel.

The effectiveness of all the stains depended upon the enzyme studied performing its normal catalytic role with the accompanying reduction, in the presence of phenazine methosulphate (PMS), of yellow, soluble methyl thiazolyl tetrazolium (MTT) to purple, insoluble formazan at the sites of enzyme activity. Staining recipes were obtained, sometimes with slight modifications, from Shaw and Prasad (1970) with the following exceptions: lactic

Table 4 Details of enzymes studied

Name	Abbreviation	Enzyme number	Method of Electrophoresis *	Comments
Glycerol-3-phosphate dehydrogenase	G3PDH	1.1.1.8	2, 4	Also abbreviated to GPD and also known as α-glycerophosphate dehydrogenase (α-GPDH)
Sorbitol dehydrogenase	SDH	1.1.1.14	4	Also abbreviated to SORDH
Lactate dehydrogenase	LDH	1.1.1.27	1, 5	
Malate dehydrogenase	MDH	1.1.1.37	2, 3, 5	Also known as NAD linked malate dehydrogenase (NAD MDH)
Malic enzyme	ME	1.1.1.40	1, 2	Also known as NADP linked malate dehydrogenase (NADP MDH)
Isocitrate dehydrogenase	IDH	1.1.1.42	3	Also abbreviated to ICD
6-Phosphogluconate dehydrogenase	6 PGD	1.1.1.44	2	Also abbreviated to 6 Pgdh and also known as phosphogluconate dehydrogenase (PGD)
Glucose-6-phosphate dehydrogenase	G6PDH	1.1.1.49	4	Also abbreviated to Gd
Superoxide dismutase	SOD	1.15.1.1	1	Also known as tetrazolium oxidase (TO)
Glutamate-oxaloacetate transaminase	GOT	2.6.1.1	3	Also known as aspartate aminotransferase (AAT)
Pyruvate kinase	PK	2.7.1.40	1, 3	
Adenylate kinase	AK	2.7.4.3	1,3	
Phosphoglucomutase	PGM	2.7.5.1	1	
Adenosine deaminase	ADA	3.5.4.4	3	
Mannose phosphate isomerase	MPI	5.3.1.8	1	
Glucose phosphate isomerase	GPI	5.3.1.9	1	Also known as phosphohexose isomerase (PHI) and phosphoglucose isomerase (PGI)

* Where more than one method was used principal method is underlined.

dehydrogenase (Lush, Cowey and Knox 1969), glycerol-3-phosphate dehydrogenase (Dando, 1970), pyruvate kinase (Brewer, 1970), adenylate deaminase (Spencer, Hopkinson and Harris, 1968) and mannose phosphate isomerase (Harris and Hopkinson, 1976). Table 5 gives details of stains. Gels were incubated in the dark until dark blue formazan bands appeared.

Not all the enzymes listed in Table 4 were examined throughout this survey; only those displaying polymorphism and easily typed from white muscle samples or yolk-sac larvae were studied consistently. The reasons for this will be discussed later (see Results, page 27). Other enzymes are known to be polymorphic in plaice (Ward and Beardmore, 1977) but are not identifiable in white muscle.

Table 5 Stain constituents: amounts quoted are sufficient to stain one slice of gel using electrophoretic methods in Table 3. Where more than one method was used for electrophoresis only quantities used for principal methods are listed.

Reagents	Enzymes															
	G3PDH	SDH	LDH	MDH	ME	IDH	6PGD	G6PDH	SOD	GOT	PK	AK	PGM	ADA	MPI	GPI
β-nicotinamide dinucleotide	4 mg	4 mg	4 mg	4 mg												
Nicotinamide dinucleotide phosphate, disodium salt					2 mg	2 mg	2 mg				4 mg	4 mg	4 mg		4 mg	2 mg
Methyl thiazolyl tetrazolium	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg	1 mg		1 mg	1 mg	1 mg	3.5 mg	3.5 mg	1 mg
Phenazine methosulphate	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg	0.5 mg		0.5 mg	0.5 mg	0.5 mg	1 mg	1 mg	1 mg
Magnesium chloride							25 mg					25 mg	25 mg		25 mg	25 mg
Ethylene diamine tetracetic acid, disodium salt	30 mg															
D. L. α-glycerophosphate, disodium salt	50 mg															
Sorbitol		50 mg														
Lithium L-lactate			30 mg													
Sodium L-malate				50 mg	50 mg											
tri-Sodium citrate						25 mg										
Manganese chloride						40 mg										
6-phosphogluconate, tri-sodium salt							10 mg									
Glucose-6-phosphate, disodium salt								10 mg								
L-aspartic acid										60 mg						
α-ketoglutaric acid										40 mg						
Pyrodoxal-5-phosphate										20 mg						
Polyvinyllpyrrolidone										60 mg						
Fast violet B										120 mg						
Phosphoenol pyruvate, trisodium salt											20 mg					
Adenosine diphosphate, disodium salt											20 mg	20 mg				
Glucose											70 mg	70 mg				
Magnesium sulphate											100 mg					
Glucose-6-phosphate dehydrogenase											25 units	25 units			25 units	25 uni
Hexokinase											10 units	10 units				
Glucose-1-phosphate, disodium salt (containing 1% glucose 1-6 diphosphate)													25 mg			
Adenosine														7.5 mg		
Sodium arsenate														25 mg		
Xanthine oxidase														0.025 units		
Nucleoside phosphorylase														0.25 units		
Mannose-6-phosphate, barium salt															10 mg	
Glucose phosphate isomerase															25 units	25 mg
Fruotose-6-phosphate, disodium salt																
0.1 M Tris (hydroxymethyl) methylamine HCL pH 7.0								20 ml		15 ml						
0.1 M Tris (hydroxymethyl) methylamine HCL pH 7.0	15 ml	15 ml		15 ml	20 ml	15 ml	15 ml				20 ml	20 ml		15 ml		
0.1 M Triethanolamine HCL pH 7.0			15 ml						20 ml				20 ml		20 ml	20 ml
2% agar in dist. H_2O	15 ml	15 ml	15 ml	15 ml	20 ml	15 ml	15 ml	20 ml	20 ml	15 ml	20 ml	20 ml	20 ml	15 ml	20 ml	20 ml

ADULT PLAICE

RESULTS

Table 6 gives details of which enzymes were tested for in each batch of live adult fish. Not all of the enzymes listed in Table 4 were examined in these samples for one or more of the following reasons : (i) the enzyme was known to be monomorphic. i.e. lactate dehydrogenase, malic enzyme, adenylate kinase.

(ii) the enzyme was not present in white muscle.

i.e. sorbital dehydrogenase.

(iii) the amounts of tissue taken from live fish were too small to produce sufficient enzyme activity for accurate resolution on starch gels, i.e. glucose-6-phosphate dehydrogenase, superoxide dismutase, glutamate-oxaloacetate transaminase, mannose phosphate isomerase.

The enzyme typing of adults in 1974 was carried out by P.R. Dando at the laboratory of the Marine Biological Association, Plymouth, as facilities were not available at Lowestoft until April, 1974; thereafter all the typing was carried out at Lowestoft.

The numbers of phenotypes found at each locus for the combined years 1974-78 are given in Table 7. The alleles are numbered throughout in order of electrophoretic mobility and Table 8 equates them with previously published systems of nomenclature. Table 9 lists the numbers of the most common phenotype found at each locus against collection ground and year, all other phenotypes are pooled; Table 10 gives a similar distribution for alleles.

The same method of statistical analysis was applied to the data for each of the enzyme systems. First the results from each sample of fish were examined; the expected numbers of each phenotype were

Table 6 Details of tests made on adult plaice.

Season	Area	Enzymes							
1974	Tea Kettle	G3PDH	MDH	PGM	GPI				
	Flamboro Off	"	"	"	"				
	Silver Pits	"	"	"	"				
	Kentish Knock	"	"	"	"				
1975	West Mud Hole	"	"	"	"	6PGD			
1976	Dover Strait	"	"	"	"	"			
	Silver Pits	"	"	"	"	"			
	West Mud Hole	"	"	"	"	"			
1977	Sole Bay	"	"	"	"	"	IDH	ADA	PK
	West Mud Hole) White Bank) Silver Pits)	"	"	"	"	"	"	"	"
1978	Dover Harbour	"	"	"	"	"	"	"	
	Smiths Knoll	"	"	"	"	"	"	"	
	Eastern Deeps) Brown Ridges)	"	"	"	"	"	"	"	

Table 7 Distribution of phenotypes in adult plaice 1974-78. Not all the possible phenotypes were found.

Phenotype	G3PDH	MDH-A	PGM	GPI-A	GPI-B	6PGD	IDH	ADA	PK
1 -1	1								
2 -2	219	268	1	320		263	104	2	34
3 -3		8	60						
4 -4			125		2			82	
6 -6					302			3	
1^a-2	2								
1 -2	27	3		21		7			1
1 -3	2		2						
1 -4			5						
2 -3	18	72	1	11		2	2		1
2 -4	2	1	1					29	
2 -6					3			6	
3 -4			154						
3 -5			2						
3 -6								1	
4 -5			1					2	
4_a-6					32			28	
5^a-6					1				
5 -6					1				
5 -7								1	
6 -7					11				
Untyped	81					16	54	6	61
Total no of fish tested	352	352	352	352	352	288	160	160	97

Table 8 The numbers given to alleles at different polymorphic loci are equated to those used elsewhere. Abbreviations in parentheses are those used by Ward and Beardmore (1977) and Beardmore and Ward (1977).

Locus	This study	Dando (1974)	Purdom et al. (1976)	Thompson et al. (1978)	Ward and Beardmore (1977)	Beardmore and Ward (1977)
G3PDH	1[a]				148	
(α-Gpdh-1)	1		1		135	7
	2[a]				113	6
	2		2		100	5
	3		3		73	4
					65	3
	4[a]				58	2
	4		4		50	1
MDH-A	1		1	1	126	3
(Mdh-2)	2		2	2	100	2
	3		3	3	77	1
	4					
PGM	1		1	1	288	8
(Pgm-1)	2		2	2	(186	7
					(171	6
	3		3	3	(148	5
					(140	4
	4		4	4	100	3
	5		5	5	(66	2
					(59	1
GPI-A	1[a]			1[a]	109	5
(Pgi-2)	1	1	1	1	104	4
	2	2	2	2	100	3
	3	3	3	3	90	2
	4	4	4	4	83	1
GPI-B	1	1	1	1	500	
(Pgi-1)	2	2	2	2	450	
	3	3	3	3	287	
	4	4	4	4	250	
	5[a]				237	
	5	5	5	5	162	
	6	6	6	6	100	
	7[a]				50	
	7	7	7	7	13	
	8				-25	
6PGD	1			1	117	
(6Pgdh)	2			2	100	
	3			3	86	
	4					
IDH	1					
(Idh-2)	2				100	
	3				88	
	4					
ADA	1				117	6
	2				108	5
	3					
	4				100	4
	5				96	3
	6				91	2
	7				80	1
PK	1					
	2					
	3					

Table 9 Phenotype distribution in adult plaice by fishing ground and year, corresponding to Table 1. Only the most common phenotype and the rest are given for each locus.

Ground	Year	Locus G3PDH Phen.2	Others	MDH-A Phen. 2	Others	PGM Phen. 4	Others	GPI-A Phen. 2	Others	GPI-B Phen. 6	Others	6PGD Phen. 2	Others	IDH Phen. 2	Others	ADA Phen. 4	Others	PK Phen. 2	Other
Tea Kettle	1974	21	3	19	5	9	15	22	2	19	5	-	-	-	-	-	-	-	-
Flamboro' Off	1974	8	1	8	1	1	8	7	2	4	5	-	-	-	-	-	-	-	-
Silver Pits	1974	23	2	19	6	4	21	21	4	25	0	-	-	-	-	-	-	-	-
	1976	6	2	11	5	8	8	14	2	14	2	14	2	-	-	-	-	-	-
Kentish Knock	1974	4	1	6	-	1	5	5	1	5	1	-	-	-	-	-	-	-	-
West Mud Hole	1975	41	3	29	15	17	27	40	4	35	9	43	1	-	-	-	-	-	-
	1976	6	-	47	14	18	43	53	8	53	8	55	2	-	-	-	-	-	-
Dover Strait	1975 NB1	-	-	5	2	1	6	7	-	5	2	4	-	-	-	-	-	-	-
Sole Bay	1976 NB2	8	1	9	-	5	4	7	2	8	1	9	-	-	-	9	-	-	-
West Mud Hole White Bank Silver Pits	1977	64	16	67	21	29	59	84	4	75	13	77	2	60	1	43	42	34	2
Dover Harbour	1977 NB3	22	10	28	6	16	18	33	1	33	1	33	1	21	-	15	16	-	-
Smiths Knoll	1978	6	8	11	3	8	6	12	2	13	1	14	-	8	1	6	8	-	-
Eastern Deeps Brown Ridges	1978	10	5	9	6	8	7	15	-	13	2	15	-	15	-	9	6	-	-

Table 10 Allele distribution in adult plaice by fishing ground and year, corresponding to Table 1. Only the most common allele and the rest are given for each locus.

Ground	Year	Locus																	
		G3PDH		MDH-A		PGM		GPI-A		GPI-B		6PGD		IDH		ADA		PK	
		Allele 2	Others	Allele 2	Others	Allele 4	Others	Allele 2	Others	Allele 6	Others	Allele 2	Others	Allele 2	Others	Allele 4	Others	Allele 2	Others
Tea Kettle	1974	44	4	43	5	30	18	46	2	42	6	-	-	-	-	-	-	-	-
Flamboro' Off	1974	17	1	16	2	9	9	16	2	13	5	-	-	-	-	-	-	-	-
Silver Pits	1974	48	2	44	6	24	26	47	3	50	0	-	-	-	-	-	-	-	-
	1976	14	2	27	5	22	10	30	2	30	2	30	2	-	-	-	-	-	-
Kentish Knock	1974	8	2	12	0	6	6	11	1	11	1	-	-	-	-	-	-	-	-
West Mud Hole	1975	85	3	72	16	57	36	84	4	79	9	87	1	-	-	-	-	-	-
	1976	12	0	107	15	69	53	114	8	114	8	112	2	-	-	-	-	-	-
Dover Strait	1975 NB 1	-	-	12	2	6	8	14	0	12	2	8	0	-	-	-	-	-	-
Sole Bay	1976 NB 2	17	1	18	0	12	6	16	2	17	1	18	0	-	-	18	0	-	-
West Mud Hole White Bank Silver Pits	1977	143	17	153	23	92	84	172	4	162	14	156	2	121	1	122	48	70	2
Dover Harbour	1977 NB 3	53	11	62	6	44	24	67	1	67	1	67	1	42	0	44	18	-	-
Smiths Knoll	1978	20	8	24	4	20	8	26	2	27	1	28	0	17	1	18	10	-	-
Eastern Deeps Brown Ridges	1978	25	5	24	6	23	7	30	0	28	2	30	0	30	0	21	9	-	-

NB1 Fish collected for 1976 experiments, therefore, included in 1976 totals.
NB2 Fish collected for 1977 experiments, therefore included in 1977 totals.
NB3 Fish collected for 1978 experiments, therefore included in 1978 totals.

calculated from observed allele frequencies according to the Hardy-Weinberg Principle of genetic equilibrium. Unfortunately due to the small size of many of the samples the numbers of individuals expected in the rarer phenotype classes were very low. Considerable pooling of the data was necessary before X^2 tests between observed and expected phenotype distributions could be carried out. In X^2 goodness of fit tests there is controversy over the permissible minimum expected values. Conventionally this has been 5.0 but recent investigations (Roscoe and Byars, 1971; Horn, 1977) suggest lower values. In this study all phenotype classes with an expected value of less than 1.0 were pooled. If this combined expected figure was still below 1.0 these classes were added to the class with the smallest expected frequency greater than 1.0. Degrees of freedom were calculated by the formula suggested by Crisp, Beaumont, Flowerdew and Vardy (1978) where the number of degrees of freedom equals $\frac{1}{2}$ (m^2-m), where m is the number of alleles. This value was reduced by one for every phenotype class removed by pooling. In many instances pooling the classes with expected values below 1.0 removed all the degrees of freedom and prevented a X^2 test.

Contingency tests were carried out on phenotype distributions between areas; expected values were pooled to comply with the views expressed by Lewontin and Felsenstein (1965) that expected values of 1 may be used in 2 x N contingency tables. When the tables were larger than 2 x N values were pooled to give minimum expecteds of 5.0. As the extent of this pooling might have obscured any genetic differences between the areas allele distributions were also compared by similar tests, although again some pooling was necessary.

Where no apparent differences between areas were found a Hardy-Weinberg test for genetic equilibrium was carried out on the overall totals.

The results for each enzyme will be described individually:

(a) Glycerol-3-phosphate dehydrogenase (G3PDH)

This enzyme did not give a strong staining reaction with the small amounts of tissue used for electrophoresis. Nevertheless one locus was identified in white skeletal muscle and this gave a simple zymogram pattern of a single band in assumed homozygotes and three bands in assumed heterozygotes. The degree of polymorphism was low and although five alleles were observed, not all were found either in every sample or even every year, see Table 11. Unfortunately in 1976, for unknown reasons, almost all the fish could not be typed.

The numbers of different phenotypes, other than the most common homozygote, in each sample were too small to allow individual analyses, see Table 9. A contingency test upon the distribution of phenotypes between areas, made possible only by pooling all classes except phenotype 2, gave a significant probability of heterogeneity (X^2 = 27.33, D.F. = 11, $0.01 > P > 0.001$), see Table 12. This was caused by significant deviations from the expected distributions in the fish collected from the West Mud Hole in 1975 and those from Smiths Knoll in 1978. A similar test upon allele distribution, following pooling of all alleles except the most common allele 2, as in Table 10, also indicated heterogeneity (X^2 = 23.42, D.F. = 11, $0.02 > P > 0.01$), are Table 13; again caused by significant differences between observed and expected distributions in the fish from West Mud Hole in 1975 and Smiths Knoll in 1978.

Table 11 Distribution of phenotypes at the G3PDH locus in adult plaice according to year. Not all the possible phenotypes were found; some rarer alleles occurred only in heterozygotes.

Phenotype	1974	1975	1976	1977	1978
1 -1	1	-	-	-	-
2 -2	56	41	12	72	38
1a-2	-	-	-	-	2
1 -2	4	1	2	9	11
1 -3	1	-	-	1	-
2 -3	1	2	-	6	9
2 -4	-	-	-	1	1
Untyped	1	-	70	8	2

Table 12 A contingency test to examine the phenotype distribution of the G3PDH locus between fishing grounds and years.

Ground	Year	Phenotype 2			Others			Totals	X^2	Probability
		Obs	Exp	D^2/E	Obs	Exp	D^2/E			
Tea Kettle	1974	21	19.39	.1337	3	4.61	.5623	24	.70	0.50 > 0.30
Flamboro' Off	1974	8	7.27	.0733	1	1.73	.3080	9	.38	0.70 > 0.50
Silver Pits	1974	23	20.20	.3881	2	4.80	1.6333	25	2.02	0.20 > 0.10
	1976	6	6.46	.0328	2	1.54	.1374	8	.17	0.70 > 0.50
Kentish Knock	1974	4	4.04	.0004	1	.96	.0017	5	.00	P > 0.99
West Mud Holo	1975	41	35.56	.8322	3	8.44	3.5064	44	4.33	0.05 > 0.02
	1976	6	4.85	.2727	-	1.15	1.1500	6	1.42	0.30 > 0.20
Sole Bay	1976	8	7.27	.0733	1	1.73	.3080	9	.38	0.70 > 0.50
West Mud Hole) White Bank) Silver Pits)	1977	64	64.65	.0065	16	15.35	.0275	80	.03	0.90 > 0.80
Dover Harbour	1977	22	25.86	.5762	10	6.14	.4266	32	3.00	0.10 > 0.05
Smiths Knoll	1978	6	11.31	2.4930	8	2.69	10.4818	14	12.97	0.001 > P
Eastern Deeps) Brown Ridges	1978	10	12.12	.3708	5	2.88	1.5606	15	1.93	0.20 > 0.10
Totals		219			52			271		

Overall X^2 = 27.33

D.F. = 11

0.01 > Probability > 0.001

Table 13 A contingency test to examine the allele distribution of the G3PDH locus between fishing grounds and years.

Ground	Year	Allele 2			Others			Totals	X^2	Probability
		Obs	Exp	D^2/E	Obs	Exp	D^2/E			
Tea Kettle	1974	44	43.04	.0214	4	4.96	.1858	48	.21	0.70> 0.50
Flamboro' Off	1974	17	16.14	.0458	1	1.86	.3976	18	.44	0.70> 0.50
Silver Pits	1974	48	44.83	.2242	2	5.17	.4318	50	.66	0.50> 0.30
	1976	14	14.35	.0085	2	1.65	.0742	16	.08	0.80> 0.70
Kentish Knock	1974	8	8.97	.1049	2	1.03	.9135	10	1.02	0.50> 0.30
West Mud Hole	1975	85	78.91	.4700	3	9.09	4.0801	88	4.55	0.05> 0.02
	1976	12	10.76	.1429	-	1.24	1.2400	12	1.38	0.30> 0.20
Sole Bay	1976	17	16.14	.0458	1	1.86	.3976	18	.44	0.70> 0.50
West Mud Hole) White Bank) Silver Pits)	1977	143	143.47	.0015	17	16.53	.0134	160	.01	0.95> 0.90
Dover Harbour	1977	53	57.39	.3358	11	6.61	2.9156	64	3.25	0.10> 0.05
Smiths Knoll	1978	20	25.11	1.0399	8	2.89	9.0353	28	10.08	0.01> 0.001
Eastern Deeps) Brown Ridges)	1978	25	26.90	.1342	5	3.10	1.1645	30	1.30	0.30> 0.20
	Totals	486			56			542		

Overall X^2 = 23.42

D.F. = 11

0.02 > Probability > 0.01

(b) Malate dehydrogenase (MDH)

Three zones of activity were observed on zymograms stained for this enzyme. The most common pattern was of a single band in each of the three zones. Variation was observed in the most anodal zone when three bands were observed, with a corresponding increase to two bands in the intermediate zone. These patterns were interpreted as the products of two loci, each controlling a dimeric molecule with intermediate hybrid bands forming between their products. Variation was only found in the most anodal form, MDH-A. The degree of polymorphism observed was not high, see Table 7, and although four alleles were found two of these were rare. Consequently the numbers of rarer phenotypes found either in each sample or each year were small, see Tables 9 and 14. X^2 tests between observed and expected phenotype distributions following application of Hardy-Weinberg were only possible for the two largest samples, one collected from the West Mud Hole in 1975 and the other from this ground and the White Bank and Silver Pits in 1977. In both instances agreement between observed and expected distributions suggested genetic equilibrium (1975 sample: $X^2 = 0.21$, D.F. = 1, $0.70 > P > 0.50$; 1977 sample: $X^2 = 1.67$, D.F. = 1, $0.20 > P > 0.10$).

A contingency test on phenotype distribution between samples, made possible by pooling all phenotypes except the common homozygote, phenotype 2, as in Table 9, showed no significant heterogeneity ($X^2 = 11.67$, D.F. = 12, $0.50 > P > 0.30$). A similar test on allele distribution, after pooling all alleles except allele 2, as in Table 10, gave the same result ($X^2 = 9.71$, D.F. = 12, $0.70 > P > 0.50$).

Table 14 Distribution of phenotypes at the MDH-A locus in adult plaice according to year. Not all the possible phenotypes were observed; rare alleles occurred only in heterozygotes.

Phenotype	1974	1975	1976	1977	1978
2-2	52	29	63	76	48
3-3	1	1	1	3	2
1-2	-	-	1	2	-
2-3	11	14	18	16	13
2-4	-	-	1	-	-

A χ^2 test between the overall observed phenotype distribution and that expected according to the Hardy-Weinberg Principle indicated genetic equilibrium ($\chi^2 = 1.57$, D.F. = 1, $0.30 > P > 0.20$).

(c) Phosphoglucomutase (PGM)

Electrophoresis of adult plaice white muscle indicated one locus controlling PGM activity. Individuals gave either two or four banded patterns, presumed to be homozygotes and heterozygotes respectively. Five pairs of bands of differing electrophoretic mobility were observed, each pair assumed to be under the control of one allele. Table 7 lists the overall numbers of phenotypes found, and Table 15 gives their distribution for each year. This latter table shows that the variability found in this enzyme in each year's potential parent fish was greater than in either of the two previously described enzymes. The numbers of phenotypes other than the most common homozygote found in each sample are listed in Table 9; similar details of allele distribution are given in Table 10.

Individual χ^2 tests between observed distributions of phenotypes and those expected according to Hardy-Weinberg were only possible for six samples, in the remainder the numbers of individuals expected in the different phenotype classes were too low for statistical analysis. However in the six possible analyses agreement between observed and expected phenotypic arrays suggested genetic equilibrium, see Table 16.

Contingency tests on both phenotype and allele distributions between the samples, made possible by pooling all individuals except either the most common homozygote, phenotype 4, or the most common allele, allele 4, respectively, as in Tables 9 and 10, showed no heterogeneity (Phenotype distribution: $\chi^2 = 20.21$, D.F. = 12,

Table 15 Distribution of phenotypes at the PGM locus in adult plaice according to year. Not all the possible phenotypes were observed; rarer alleles only occurred in heterozygotes.

Phenotype	1974	1975	1976	1977	1978
2-2	1	-	-	-	-
3-3	8	6	12	26	8
4-4	15	17	27	34	32
1-3	-	-	1	1	-
1-4	-	1	2	-	2
2-3	1	-	-	-	-
2-4	1	-	-	-	-
3-4	38	20	40	35	21
3-5	-	-	2	-	-
4-5	-	-	-	1	-

Table 16 The results of X^2 tests carried out between observed and expected phenotype distributions in six samples. Such tests were not possible for the remaining samples.

Area	Year	X^2	D.F.	Probability
Flamboro' Off	1974	2.70	1	0.10> 0.05
Silver Pits	1974	3.95	2	0.20> 0.10
Kentish Knock	1974	.67	1	0.50> 0.30
West Mud Hole	1975	.59	1	0.50> 0.30
Dover Harbour	1977	2.37	1	0.20> 0.10
Smiths Knoll	1978	1.27	1	0.30> 0.20

$0.10 > P > 0.05$; allele distribution : $X^2 = 16.77$, D.F. = 12, $0.20 > P > 0.10$).

A X^2 test between the overall observed and expected phenotype distributions following application of the Hardy-Weinberg Principle indicated genetic equilibrium ($X^2 = 4.90$, D.F. = 4, $0.30 > P > 0.20$).

(d) Glucose phosphate isomerase (GPI)

Three zones of activity were observed on zymograms stained for this enzyme. These have been interpreted as the products of two loci, each controlling a dimeric molecule, with a third, intermediate, zone caused by hybrid molecules (Dando, 1974). The results of this study agreed with this interpretation. Polymorphism was found at both loci, homozygotes with single bands and heterozygotes with three. The behaviour of the bands in the intermediate zone when polymorphism was present at either the faster GPI-A locus or the slower GPI-B locus was consistent with the above interpretation. However in some individuals which were polymorphic at both loci, only three bands were observed in this intermediate zone although four molecular hybrids could be formed. This deficiency was assumed to be due to overlapping caused by two of the four hybrid molecules having equal migration rates through the gel.

Table 7 gives the overall distribution of phenotypes; not all known alleles were observed. In every year the numbers of different phenotypes apart from the most common homozygote were low at both loci, see Table 17. Details of the number of rare phenotypes found in each sample aregiven in Table 9; no statistical analysis of individual samples was possible. Details of the number of rare alleles found in each sample aregiven in Table 10.

Table 17 Distribution of phenotypes at the GPI-A and GPI-B loci in adult plaice according to year. Not all the possible phenotypes were observed and rare alleles occurred only in heterozygotes.

Phenotype	1974	1975	1976	1977	1978
(i) GPI-A					
2 -2	55	40	74	91	60
1 -2	7	1	7	4	2
2 -3	2	3	3	2	1
(ii) GPI-B					
4 -4	1	-	-	1	-
6 -6	53	35	72	83	59
2 -6	-	1	1	1	-
4 -6	6	7	7	9	3
5^a-6	-	-	1	-	-
5 -6	1	-	-	-	-
6 -7	3	1	3	3	3

The low levels of polymorphism prevented contingency tests of either phenotype or allele distribution between samples being made for either locus. Even the numbers of rare phenotypes in the overall distribution for either locus were too low for X^2 tests to be made between the observed distributions and those expected according to Hardy-Weinberg although no apparent differences between these distributions was found.

(e) 6 - phosphogluconate dehydrogenase (6PGD)

A single locus was found in plaice white muscle. Again this enzyme had a dimeric structure, single-banded homozygotes, three banded heterozygotes. This enzyme was not studied in 1974, but was included in the other years. Polymorphism was very low, see Table 7, and no statistical analysis was possible, see Tables 9, 10 and 18.

(f) Iso-citrate dehydrogenase (IDH)

Again only a single locus was found. Tests for this enzyme were only made in 1977 and 1978. Only two heterozygotes were observed and a substantial number of fish were untyped due to poor gel resolution, see Table 7. The heterozygotes hade three bands, again indicating a dimeric molecule. No statistical analysis was possible.

(g) Adenosine deaminase (ADA)

A single locus was present in plaice, with a monomeric molecular structure, i.e. single banded homozygotes and two banded heterozygotes. Only the fish collected in 1977 and 1978 were tested for this enzyme. Polymorphism was higher than in any other enzyme used, except PGM, see Table 7. Six alleles were found, although not all of them in both years, see Table 19. The numbers

Table 18 Distribution of phenotypes at the 6PGD locus in adult plaice according to year. Not all phenotypes were observed and rare alleles occurred only in heterozygotes with allele 2.

Phenotype	1974	1975	1976	1977	1978
2-2	Not	43	73	86	62
1-2	tested	1	3	2	1
2-3	-	-	1	-	-
Untyped	-	-	7	9	-

Table 19 Distribution of phenotypes at the ADA locus in adult plaice in 1977 and 1978. Not all possible phenotypes were observed and rare alleles only occurred in heterozygotes.

Phenotype	1977	1978
2-2	2	-
4-4	52	30
6-6	2	1
2-4	21	8
2-6	2	4
3-6	-	1
4-5	1	1
4-6	14	14
5-7	-	1
Untyped	3	3

of phenotypes and alleles, apart from the common homozygote, phenotype 4, and the common allele, allele 4, found in each sample are given in Tables 9 and 10. Three of the samples were too small to be tested for genetic equilibrium but in the remaining two, collected from the West Mud Hole, White Bank and Silver Pits areas of the North Sea in 1977 and from Dover Harbour, also in 1977 but for use in the 1978 breeding season, compliance between observed and expected phenotype distributions was found (X^2 = 0.35, D.F. = 2, $0.90 > P > 0.80$ in the former; X^2 = 0.94, D.F. = 3, $0.90 > P > 0.80$ in the latter).

Contingency tests on both phenotype and allele distributions between samples, made possible by pooling all phenotypes except the most common homozygote, phenotype 4, and all alleles except the most common allele, allele 4, suggested homogeneity (Phenotype distribution: X^2 = 9.32, D.F. = 4, $0.10 > P > 0.05$; allele distribution: X^2 = 7.97, D.F. = 4, $0.10 > P > 0.05$).

The overall phenotype distribution for all the samples did not deviate significantly from Hardy-Weinberg expectations, (X^2 = 0.64, D.F. = 3, $0.90 > P > 0.80$).

(h) Pyruvate kinase

Tests were made for this enzyme in 1977 only. One locus was found, with a dimeric molecular structure. Only two heterozygotes were observed out of 36 fish typed with 61 fish untyped, see Table 7 ,due to weakness of staining density and poor resolution. Tests were not repeated in 1978. No statistical analysis was possible.

DISCUSSION

Each enzyme studied in the rearing experiments will be discussed individually.

(a) G3PDH

The first electrophoretic identification of this enzyme was made by Sims (1965) who found neither inter nor intraspecific differences in the two species of Drosophila he studied. Interspecific variation in Drosophila was reported by Hubby and Throckmorton (1965) and intraspecific variation discovered by Grell (1967) in D. melanogaster. In the latter, polymorphism occurred at one locus with two alleles controlling a dimeric molecule.

The first electrophoretic study of G3PDH in fish was made by Nyman (1967) who described single and five banded tissue specific patterns in the Atlantic salmon, Salmo salar. He suggested that these were two forms of the enzyme each controlled by a separate locus and existing as a tetrameric molecule with molecular hybrids causing the intermediate bands. Genetic polymorphism in fish was reported in 1970 in the Pacific ocean perch, Sebastes alutus (Johnson, Utter and Hodgins), the megrim, Lepidorhombus whiffiagonis (Dando) and the skipjack tuna, Katsuwomis pelamis (McCabe, Dean and Olson). These authors found polymorphism at a single locus in skeletal muscle; single and three banded phenotypes suggested a dimeric molecular structure with the middle band representing a hybrid molecule. More recently this enzyme has been studied in the herring, Clupea harengus, the smelt Osmerus esperlanus, the brown trout, Salmo trutta, the rainbow trout, Salmo gairdneri (Engel, Schmidtke and Wolf, 1971a) the saury, Cololabis saira (Numachi, 1971) and the lake whitefish, Coregonus

clupeaformis (Clayton, Franzin and Tretiak, 1973a). The results indicated the existence of three loci in Teleosteans, with tissue specificity. It is not possible to relate different loci to specific tissues using the data of Engel et al. (1971a) but Numachi (1971) reported only one locus in white skeletal muscle whilst two loci were found to be active in this tissue in the lake whitefish (Clayton, et al., 1973a). Whenever polymorphism was found at a locus the electrophoretic patterns indicated a dimeric structure for the G3PDH molecule. Clayton et al. (1973a) confirmed this interpretation in lake whitefish by demonstrating by breeding experiments the inheritance of proposed different alleles at the two loci active in white muscle. Utter and Hodgins (1972) reported only one polymorphic locus in skeletal muscle of rainbow trout and later confirmed the genetical control at this locus by family studies (Utter, Hodgins, Allendorf, Johnson and Mighell, 1973b). The other two loci described by Engel et al. (1971a) in this species were not observed but in further studies on rainbow trout Allendorf, Utter and May (1975) reported a second locus in muscle without polymorphism but forming hybrid bands with products of the locus described initially. Single polymorphic loci controlling a dimeric molecule have been described in skeletal muscle of several other fish including two salmonoids, the pink salmon, Oncorhynchus gorbusha (Aspinwall, 1973) and the cutthroat trout, Salmo clarki (Reinitz, 1977a), the cod, Gadus morhua (Cross and Payne, 1975), a pleuronectid, the starry flounder, Platichthys stellatus (Johnson and Beardsley, 1975) and four more marine species, the walleye pollock, Theragra chalcogramma the sable fish, Anopoploma fimbria and two

pleuronectids, the flathead sole, Hippoglossoides elassodon and the Greenland turbot, Reinhardtius hippoglossoides (Johnson, 1977). Similar results have been found in more general studies within the family Scorpaenidae (Johnson, Utter and Hodgins, 1972), cavefish of the genus Astyanax (Avise and Selander, 1972) and pupfish of the genus Cyprinodon (Turner, 1974). A single polymorphic locus was also reported in skeletal muscle of the brown trout, (Allendorf, Ryman, Stennak and Stahl, 1976) but more recently two more monomorphic loci have been described in this species (Allendorf, Mitchell, Ryman and Stahl, 1977), one in muscle and the other in liver. These three loci correspond to those described by Engel et al. (1971a). Wheat, Whitt and Childers (1973) described two loci, each coding for for a dimeric molecule in interspecific sunfish hybrids of the genus Lepomis, one active in liver and the other in skeletal muscle: Buth (1977) also reported two dimeric loci in redhorse suckers of the genus Moxostoma but both active in muscle. Ward and Galleguillos (1978) found two loci in the dab, Limanda limanda, and the flounder, Platichthys flesus; one polymorphic and expressed in muscle and the other monomorphic and active in the liver.

Although the reported number of loci controlling G3PDH varies between species, presumably due to differences in evolutionary history or in experimental methodology, in all instances except the report by Nyman (1967) the evidence suggests a dimeric structure for the G3PDH molecule. This appears to be the case in plaice; a single dimeric locus was found in skeletal muscle in this study. Ward and Beardmore (1977) typed two loci in plaice, one active in liver and the other in muscle. The G3PDH locus discussed here is referred to as α-Gpdh-1 by these authors.

In this study the degree of polymorphism for G3PDH found in the adult plaice was low. Five out of the seven known alleles were observed but the majority of the fish were homozygous for the most common allele 2. The variety of geographical sources, the small numbers of fish from each area and the very low level of polymorphism make comparisons between samples and areas very difficult. The results, however, do show differences in the distribution of phenotypes and alleles between samples. Whilst this may be caused by genetic differences between certain spawning grounds one of the two samples deviating significantly from the other North Sea samples was from a locality represented in another year by a different sample where no such deviation was observed. The possibility of genetic differences occurring between different spawning grounds will be examined more fully in the analysis of the data obtained from the egg samples collected in different areas.

It has been suggested (Purdom et al. 1976) that null alleles may be present at this locus. A review of null alleles and the possible cause of their occurrence was presented by Manwell and Baker (1970). They gave no examples in fish, but null alleles have since been found for lactate dehydrogenase in carp (Engel, Schmidtke, Vogel and Wolf, 1973) and for isocitrate dehydrogenase in hybrids between Micropterus species (Wheat, Childers and Whitt, 1974). In marine flatfish Dando (pers. comm.) has observed null alleles in G3PDH in the flounder. However no evidence of null alleles was found in any of the plaice typed from 1975 to 1978; Ward and Beardmore (1977) made no mention of null alleles at this locus in plaice.

(b) MDH

NAD linked malate dehydrogenase exists within cells in two distinct forms: one free in the cytoplasm and the other tightly

bound to the mitochondria. Each produces a characteristic electrophoretic pattern (Markert and Muller, 1959; Thorne, Grossman and Kaplan, 1963). The present study is concerned only with the former, the cytoplasmic or supernatant form of MDH.

Genetic polymorphism in this enzyme was first described in human red blood cells (Davidson and Cortner, 1967). They reported the existence of two alleles at a single locus controlling a dimeric molecule. Numachi (1970) claims to present the first report of genetic variation in fish in the saury, C. saira. He reported the presence of two loci controlling MDH in skeletal muscle, each coding for a different dimeric molecule but also producing hybrid bands. Polymorphism at one of the loci supported this interpretation. However Bailey, Cocks and Wilson (1969) had already reported two similar forms of supernatant MDH in the king or chinook salmon, Oncorhynchus tshawytscha and the rainbow trout, S. gairdneri. In a more detailed publication, Bailey, Wilson, Halver and Johnson (1970) reported these two forms of supernatant MDH in a range of fish species; the only exception to dual control was the lungfish, Eptatetrus stouti, where only one locus was involved. The remaining species which included five more salmonids, the golden trout, Salmo aguabonita; the brown trout, S. trutta; the brook trout, Salvelinus fontinalis; the silver or coho salmon, Oncorhynchus kisutch and the sockeye salmon, Oncorhynchus nerka and nine other species, the dogfish, Squalus acanthias; the chimaera, Hydrolagus colliei; the green sturgeon, Acipenser medirostris; the white sturgeon, Acipenser transmontanus; the cod, Gadus callarias (morhua), the starry flounder, P. stellatus; the smelt, Spirinchus sp; the shad,

Alosa sapidissima and the herring, C. harengus, all showed two forms of supernatant MDH with differing electrophoretic mobilities. In the majority of species the control of MDH was as previously described; single loci coding for each of the two dimeric forms with the formation also of intermediate hybrid bands. In salmonids, however, each form of MDH was apparently under the control of more than one locus as polymorphism in either form gave differential staining of isozymes; family studies and biochemical analyses led to the suggestion that four alleles at two loci control each dimeric MDH sub-unit. Further investigations in salmonids have supported this interpretation (the king or chinook salmon (Utter and Hodgins, 1972), the chum salmon, Oncorhynchus keta (Numachi, Matsumiya and Sato, 1972), the pink salmon, O. gorbuscha (Utter, Allendorf and Hodgins, 1973a, Aspinwall, 1974), the rainbow trout (Utter et al., 1973a, Allendorf et. al. 1975), the masu salmon, Oncorhynchus masou (Utter et al., 1973a), the brown trout (Allendorf et. al. 1976, Allendorf, et. al. 1977), the cutthroat trout, S. clarki (Reinitz, 1977a), and the lake whitefish, C. clupeaformis (Franzin and Clayton, 1977).

The simpler control of MDH as reported in non-salmonid fish species by Bailey et al. (1970) i.e. two dimeric sub units controlled by single loci, has been widely confirmed in teleosts. The range examined includes the killifish, Fundulus heteroclitus (Whitt, 1970), interspecific hybrids of bass, Micropterus spp. (Wheat, Childers, Miller and Whitt, 1971) hybrids between bluegills, Lepomis spp. (Wheat, Whitt and Childers, 1972) the walleye, Stizostedion vitreum vitreum (Clayton, Tretiak and Kooyman, 1971)

cavefish of the Astyanax genus (Avise and Selander, 1972), four species of darters, genus Etheostoma (Martin and Richmond, 1973), three Sebastes spp. (Johnson, Utter and Hodgins 1973), the sauger, Stizostedian canadense (Clayton, Harris and Tretiak, 1973b), seven Xiphophorus species of platyfish and swordtails (Scholl and Anders, 1973), eight shiners, Notropis spp. (Rainboth and Whitt, 1974), five athernid Menidia spp. (Johnson, 1975) and in five pleuronectids, three from the Pacific, Microstomus pacificus (called the Dover sole but not to be confused with Solea solea), the flathead sole, H. elassodon and the rock sole, Lepidopsetta belineata, (Johnson, 1977), and two European species the dab, L. limanda, and the flounder, P. flesus (Ward and Galleguilos, 1978). Frydenberg and Simonsen (1973) reported two loci in the viviparous blenny, Zoarces viviparus, but deduced from their five banded electropherograms that the MDH molecule had a tetrameric structure. They equated their results to those of Bailey et al. (1970) and Wheat et al. (1971); the reasons for this are unclear since these authors describe a dimeric molecular structure for supernatant MDH.

The plaice, in common with the majority of species listed above, has a single locus coding for each form of supernatant MDH. Both forms are identifiable in skeletal muscle. Genetic polymorphism has been found in only the most anodal MDH-A form (Purdom et al. 1976). Ward and Beardmore (1977) confirmed this polymorphism and called the locus Mdh-2.

Although the degree of polymorphism found at the MDH-A locus in the adult plaice typed in this project was slightly higher than for G3PDH in the same fish, the majority of individuals, 76%, were homozygous for the most common allele 2. Three other alleles were

observed but only one, allele 3, was found in appreciable numbers. Of the remaining alleles only three examples were found of allele 1, and a single example of allele 4; in all four instances these rare alleles were observed in heterozygotes with the common allele 2. X^2 tests upon observed and expected phenotype distributions were only possible for two samples and genetic equilibrium was indicated in both. Contingency tests on phenotype and allele distributions between all the samples suggested homogeneity and a X^2 test upon overall observed and expected phenotype distributions indicated genetic equilibrium.

With respect to the MDH-A locus differences between North Sea spawning grounds do not appear to exist.

(c) PGM

The first reported identification of PGM isozymes by electrophoresis was by Roberts and Tsuyuki (1963) in muscle extracts of the rainbow trout, S. gairdneri. The number of isozymes observed in a sample increased from two to five as the pH of the gel buffer was raised from 8.0 to 9.0. No differences between individuals were described and no attempt was made at genetic interpretation.

The existence of genetic polymorphism in PGM was first demonstrated by the electrophoresis of human red blood cell lysates (Spencer, Hopkinson and Harris, 1964). A single locus was described controlling two banded homozygotes and four banded heterozygotes. In further reports (Hopkinson and Harris, 1965, 1966 and 1968) two more loci were identified in human red cell lysates, each controlling a similarly expressed polymorphism. Family data supported their interpretation of one allele controlling two isozymes and Spencer et al. (1964) suggested that these may be the phosphorylated and dephosphorylated forms of the PGM molecule. However this hypothesis

was disproved by Dawson and Mitchell (1969) who separated these two forms and found no electrophoretic difference between them. They considered that the double banded homozygous pattern was caused by conformational isomers of the same enzyme molecule.

PGM polymorphism in fish was first identified in the Atlantic herring, Clupea harengus harengus (Lush, 1969). Two loci were found in liver extracts, one monomorphic and one polymorphic with three alleles controlling single banded homozygotes and two banded heterozygotes. Roberts, Wohnus and Ohno (1969) also reported a single polymorphic locus in rainbow trout but with two other monomorphic loci present. Varied results have been reported since in other salmonids; a single polymorphic locus in some species (the sockeye salmon, O. nerka (Utter and Hodgins, 1970), the coho salmon, O. kisutch (Utter, et al. 1973a) and the golden trout, S. aguabonita (Gall, Busack, Smith, Gold and Kornblatt, 1976)) and a single monomorphic locus in others (the pink salmon, O. gorbuscha and the chum salmon, O. keta (Utter et al., 1973a)). Originally a single monomorphic locus was described in the cutthroat trout, S. clarki, (Utter et al., 1973a) but Reinitz (1977a) in a more detailed report found this locus to be polymorphic. In the chinook salmon, O. tshawytscha, no polymorphism was found but an extra isozyme was present which Utter et al. (1973a) suggested was either a conformational isozyme or the product of a duplicate locus. More recently a single polymorphic locus has been reported in this species (Kristiansson and McIntyre, 1976). However two loci, electrophoretically distinct but both monomorphic have been found in the brown trout, S. trutta, (Allendorf, et al. 1977).

For other teleosts results similar to those of Spencer et al. (1964) have been described (the mackerel, Scomber scombrus, the striped bass, Rossus saxatilus, the brook trout, S. fontinalis the sword fish Xiphias gladius (Dawson and Jaegar, 1970) and the loach, Misgurnus anguillicaudatus (Kimura, 1976)). Two and four banded phenotypes were again interpreted as homozygotes and heterozygotes, respectively, with the extra bands caused by conformational isomers. Smith and Jamieson (1978) observed more complex isozyme patterns in the mackerel, S. scombrus; two loci were described but only one, in skeletal muscle, was routinely typed, again showing two and four banded phenotypes. Complicated electropherograms had also been produced from red blood cell lysates of the cod, G. morhua, (Tills, Mourant and Jamieson, 1971) and although the isozymes could be divided into two zones more accurate typing and genetical interpretation proved impossible.

The simple isozyme patterns found in the Atlantic herring (Lush, 1969) i.e. single banded homozygotes and double banded heterozygotes, was also found in the Pacific herring, Clupea harengus pallasi (Utter, 1970). More recently this type of variation has been described in the Scorpaenidae family, mainly in Sebastes species (Johnson, Utter and Hodgins, 1971, 1972), cavefish, genus Astyanax (Avise and Selander, 1972), sunfish of the family Centrarchidae (Avise and Smith, 1974; Avise, Straney and Smith, 1977), the killifish, F. heteroclitus (Mitton and Koehn, 1975), New World cichlids (Kornfield and Koehn, 1975) and the sable fish, A. fimbria (Johnson, 1977). Hjorth (1971) reported similar polymorphism in the viviparous blenny, Z. viviparus but found three loci coding for PGM in brain extracts.

In pleuronectids PGM polymorphism has been reported in the starry flounder, P. stellatus (Johnson and Beardsley, 1975), the arrowtooth flounder, Atheresthes stomias, the rex sole, Glyptocephalus zachirus, the flathead sole, H. elassodon, the rock sole, L. belineata, the Pacific halibut, Hippoglossus sterolepsis and the long nose dab, Limanda probascidea (Johnson, 1977). Single polymorphic loci were mentioned but no descriptions were presented. Purdom et al. (1976) described a single polymorphic locus in the skeletal muscle of the plaice; again with two banded homozygotes and four banded heterozygotes. In contrast Ward and Beardmore (1977) identified two PGM loci in '0' group plaice but only one in muscle samples from larger fish. This latter locus, referred to as Pgm-1 by Ward and Beardmore (1977) is the one described by Purdom et al., (1976) and is also the subject of this study. However the electrophoretic method used by Ward and Beardmore (1977) produced single banded homozygotes and double banded heterozygotes. A comparison made in this laboratory by testing the same individuals by both techniques proved that the same polymorphism was being identified although isozyme patterns and migration rates were different. This locus was also used by Beardmore and Ward (1977) in their study of changes in heterozygosity levels with time in juvenile plaice populations. Since then, Ward and Galleguillos (1978) have reported similar polymorphism in two more pleuronectids, the dab, L. limanda and the flounder, P. flesus.

In the fish examined here the degree of polymorphism observed at the PGM locus was higher than at the two previously discussed loci. In this study five alleles have been identified at this locus and all five were found in the adult fish. Ward

and Beardmore (1977) describe seven alleles at this locus. X^2 tests between observed numbers of phenotypes and those expected according to Hardy-Weinberg were possible for six of the samples; compliance with the hypothesis of genetic equilibrium was found in all six. Contingency tests to compare both phenotype and allele distributions between samples showed no significant deviation from uniformity. The overall phenotype distribution did not deviate from Hardy-Weinberg expectations. No differences between spawning areas of the North Sea appear to exist with respect to this locus.

(d) GPI

Polymorphism in GPI was first described in the mouse, Mus musculus (Carter and Parr, 1967). Single and three banded patterns were observed. Family data showed these to represent homozygotes and heterozygotes respectively, indicating that in mice the GPI molecule exists as a dimer and that variation is controlled by alleles at a single locus. Similar control of GPI has been found throughout a wide range of vertebrates and invertebrates; see Avise and Kitto (1973) for a review of species examined. However discrepancies were found in the characid fish, Astyanax mexicanus (Avise and Selander, 1972) and in the viviparous blenny, Z. viviparus (Yngaard, 1972) where two forms of GPI each controlled by a separate locus were identified. Hybrid zones typical of multilocus control of dimeric molecules were found. These two latter forms of GPI were found in the majority of species tested in more extensive examinations in bony fish involving 82 species in 46 families (Avise and Kitto, 1973; Dando, 1974; and Schmidtke, Dunkhase and Engel, 1975). Table 20 gives details of the results.

Table 20 The number of loci controlling GPI in 82 species of bony fish

	No. of loci			
	1	2	3	4
No. of species	8 (3 spp. of primitive Holosteans; 4 spp. of clupeids inc. the Atlantic herring, C. harengus harengus, the pilchard, Sardina pilchardus and the sprat, Sprattus sprattus, and the Northern pipefish Syngnathus fuscus)	68	5 (4 spp. of salmonid and the tetraploid barb, Barbus barbus)	1 (the tetraploid goldfish Carassius gibelio auratus)

Two loci controlling two forms of GPI were also reported in more detailed specific studies carried out upon three species of rockfish, genus Sebastes (Johnson, et.al. 1973), the family Centrarchidae (Avise and Smith, 1974; Whitt, Childers, Shaklee and Matsumoto, 1976; Champion and Whitt, 1976; Avise et.al. 1977), two catostomids, Moxostoma spp. (Buth, 1977) and three species of the Cyprindae (Child and Solomon, 1977). When large numbers of individuals of a species were examined genetic polymorphism was frequently found at one or both loci. An individual with apparently three loci coding for GPI was found in a total of 118 specimens of Cheirodon axelrodi, a Characid fish (Kuhl, Schmidtke, Weiler and Engel, 1976); the remaining 117 fish had two loci, both polymorphic. The authors suggested that this third form of GPI was the product of a hybrid gene locus caused by an unequal crossover between two closely linked GPI loci, although the possibility of triploidy remains.

In salmonids three GPI loci had been found in four of the five species examined by Avise and Kitto (1973), Dando (1974) and Schmidtke et. al. (1975). These were the rainbow trout, S. gairdneri, the brown trout, S. trutta, the brook trout, S. fontinalis and the powan, Coregonus laveretus; the exception was the Atlantic salmon, S. salar, where only two loci were found. Conflicting results were originally presented for the brown trout, Avise and Kitto (1973) reported three loci whilst Schmidtke et al. (1975) reported two but in a later more extensive study Allendorf, et. al., (1977) confirmed the presence of three loci. In all these studies all the individuals were monomorphic for all loci. Ferguson (1974) attempted to use GPI to differentiate between species of United Kingdom coregonids but all the fish gave the

same undescribed pattern; similarly Kristiansson and McIntyre (1976) examined chinook salmon O. tshawytscha, but could not interpret the results obtained. However Child (1977) and Reinitz (1977a) studying the char, Salvelinus alpinus, and the cutthroat trout, S. clarki, respectively, both reported three monomorphic GPI loci.

In pleuronectids two loci have been reported in the Norwegian topknot, Phrynorhombus norvegicus, the dab, L. limanda, the flounder, P. flesus and the Dover sole, S. solea (Dando, 1974) and the arrowtooth flounder, A. stomias (Johnson, 1977). In the plaice Dando (1974) described two polymorphic GPI loci, each controlling a dimeric molecule and Purdom et al. (1976) and Ward and Beardmore (1977) confirmed this; the former with family data. Beardmore and Ward (1977) used one of these loci, referred to by them as Pgi-2 and in this study as GPI-A, in their study of changes in multi-locus heterozygosity with age in juvenile plaice.

Considering the results obtained in this study at the faster migrating of the two loci, GPI-A, polymorphism was very low. Only three alleles were found and in only three of the possible six genotype combinations. The majority of the individuals (91%) were homozygous for the most common allele 2 whilst the remaining fish were heterozygotes of this allele and either allele 1 or 3. Although two other alleles are known to exist at this locus they were not observed in these fish. Polymorphism at this locus was lower than in any of the enzymes discussed so far; consequently statistical tests could not be carried out.

Polymorphism at the second locus, GPI-B, although still low was higher than at the GPI-A locus. Six alleles were found, combining to form seven genotypes. The majority of individuals, 85%, were homozygous for the most common allele 6 whilst the remainder, apart from two fish homozygous for allele 4, were all heterozygotes of this allele and one of the rarer alleles. Although ten alleles have been identified at this locus four were not found in these fish. As with the previous locus, statistical analysis was not possible due to the level of polymorphism.

A more detailed analysis of the presence or absence of any geographical differences in genetic variation with respect to either of the GPI loci will be made later in this thesis using the data obtained from egg samples collected from different spawning grounds.

(e) 6 PGD

6 PGD was first detected electrophoretically in human red cell lysates (Fildes and Parr, 1963). Polymorphic forms and their mode of inheritance were described but the dimeric structure of the 6 PGD molecule was not shown until Parr (1966) resolved single banded homozygotes and triple banded heterozygotes. Family studies using laboratory rats demonstrated control of the enzyme by a single autosomally inherited locus. In fish similar results were first described in the diploid barb, Barbus tetrazona (Bender and Ohno, 1968). However in two other presumed tetraploid Cyprinid species, the carp, Cyrinus carpio, and the goldfish, Carassius gibelio auratus, two loci were found, with polymorphism at only one locus in the latter species. A single 6 PGD locus controlling a dimeric molecule, as in B. tetrazona, has been found in the

majority of species examined. These include the skipjack tuna K. pelamis (McCabe et. al., 1970) the cavefish, A. mexicanus (Avise and Selander, 1972), the viviparous blenny, Z. viviparus (Yngaard, 1972), bluegills and sunfish of the family Centrarchidae (Wheat et. al., 1973; Whitt, Childers and Cho, 1973a; Avise and Smith, 1974; Avise et.al., 1977), Xiphophorus spp. (Scholl, 1973), the pink salmon, O. gorbuscha, the chum salmon, O. keta, the sockeye salmon, O. nerka, the coho salmon, O. kisutch, the chinook salmon, O. tshawytscha and the rainbow trout, S. gairdneri (Utter, Allendorf and Hodgins, 1973a), the golden trout, S. aguabonita, (Gall et. al., 1976) and the loach, M. anguillicaudatus (Kimura, 1976). Two monomorphic loci were reported in the brown trout, S. trutta, (Allendorf et. al., 1977) and in two tetraploid catostomid Moxostoma species (Buth, 1977). In plaice Ward and Beardmore (1977) described a single polymorphic locus, 6-Pgdh, which corresponds to the locus studied in this project.

In this project this enzyme was not examined in all years, only from 1975 onwards. Three alleles were observed although four are known to exist in plaice. Over 96% of the tested fish were homozygous for allele 2 whilst the other two alleles typed were only found in heterozygotes in combination with allele 2. The infrequency of polymorphism at this locus prevented any statistical analysis. Although the possibility of carrying out inheritance studies with heterozygous parents at this locus was slight, tests continued to be made for this enzyme in 1976, 1977 and 1978, mainly because little extra effort was involved in staining for 6 PGD upon gels run to test for G3PDH and NAD. MDH.

(f) IDH

Henderson (1965) reported the first electrophoretic investigation of NADP.IDH in the mouse, M. musculus. Two forms were identified, one found mainly in the mitochondrial fraction and the other in the supernatant fraction of tissue homogenates. Each form was controlled by a single locus. Polymorphism, of such a type as to indicate a dimeric molecular structure, was found in the supernatant IDH.

The first description of such different forms of IDH in fish was made by Quiroz-Gutierrez and Ohno (1970) who found similar genetical control of IDH in the surf smelt, Hypomesus pretiosus. However in two other species these authors found exceptions from the usual vertebrate pattern of single loci coding for the mitochondrial (m) and the supernatant (s) forms of IDH. In the goldfish, C. gibelio auratus, and the carp, C. carpio, both the m-IDH and the s-IDH were found to be controlled by two loci, with the products of each locus being separated electrophoretically. In the goldfish polymorphism was found at one of the s-IDH loci and in the carp at both of them resulting in three banded double homozygotes, six banded single heterozygotes and ten banded double heterozygotes; electrophoretic patterns typical of the combining dimeric products of two loci. These results support the hypothesis that these two species evolved by tetraploidization.

Reports made since then of NADP.IDH in fish may be divided into two groups: firstly, those showing results similar to those of Quiroz-Gutierrez and Ohno (1970) where the different forms of IDH and the numbers of loci involved was as described and, secondly,

those describing more simple accounts of the observed numbers of loci and the absence or presence of polymorphism with, in some instances, mention of tissue specificity. Considering the former group another comparison between diploid and reputed tetraploid species was made by Wolf, Engel and Faust (1970). Single polymorphic loci were found to control m-IDH and S-IDH in the Atlantic herring, C. harengus harengus, whilst in the rainbow trout, S. gairdneri, two loci, one polymorphic, coded for a m-IDH but for the s form only a single locus was described. Polymorphism at this locus was interpreted by assuming tetrasomic inheritance of s-IDH. The same authors (Engel, Faust and Wolf, 1971b) were able to differentiate between m-IDH in diploid and tetraploid Cyprinids, a single monomorphic locus was found in the former and two monomorphic loci were found in the latter, but in both diploid and tetraploid species two loci, one polymorphic, controlled s-IDH. Similarly, breeding experiments between diploid Xiphophorus species (Siciliano and Wright, 1973) proved that in this genus a single locus controls m-IDH and two loci control s-IDH. Thus with respect to s-IDH the relationship between diploid and tetraploid species in not simple. Further reports have all concerned salmonids. Conflicting accounts of s-IDH inheritance in rainbow trout have been published. From family studies Ropers, Engel and Wolf (1973) suggested the existence of at least two separate s-IDH loci but could only interpret all their results by assuming disomic inheritance in some instances and tetrasomic in others. More recent work (Allendorf and Utter, 1973; Allendorf, et. al., 1975) only identified genetic control by two disomic loci and further family studies (Reinitz, 1977b) supported this interpretation; additionally this author differentiated between those two s-IDH loci found in liver and two

more s-IDH loci specific to muscle. In contrast in two other salmonids, the pink salmon, O.gorbuscha, and the chum salmon, O. keta, only single s-IDH loci were found in liver (May, Utter and Allendorf, 1975), with polymorphism in the latter species. Similarly single loci were found in the liver and muscle extracts of the brown trout, S. trutta (Allendorf et. al., 1977) but lack of polymorphism prevented any identification of duplicate loci as found in rainbow trout.

More simple observations have been made in a variety of species. A single monomorphic locus has been reported in 31 species of the family Scorpaenidae (Johnson et. al., 1972, 1973) and a single polymorphic locus is claimed in the viviparous blenny, Z. viviparus (Frydenberg and Simonson, 1973), Centrarchid sunfish (Avise and Smith, 1974, Wheat et al., 1974; Avise et al., 1977) and the walleye pollack, T. chalcogramma (Johnson, 1977). In the viviparous blenny, in contrast to any other species examined, s-IDH was found to be a monomeric molecule, i.e. with single banded homozygotes and double banded heterozygotes. Two loci, one monomorphic and one polymorphic have been described in the cavefish, A. mexicanus (Avise and Selander, 1972). Cross and Payne (1976) described two loci in the cod, G. morhua one present in liver and the other in heart possibly s-IDH and m-IDH respectively, with polymorphism at the former. A mention was made by Yardley and Hubbs (1976) that IDH was monomorphic in two mosquito fish, Gambusia spp., but no details of number of loci nor tissues examined were given. In plaice, two loci have been described (Ward and Beardmore, 1977), one found in liver and the other in muscle; both were polymorphic but the former to a much

greater degree. It is the latter locus which has been examined in this study. As the usual practice was to place small white pieces of tissue directly into the starch gels to leach out soluble proteins, the results for this locus presumably relate to a form of s-IDH.

In this study this enzyme was tested for only in 1977 and 1978. The electrophoretic results were difficult to interpret due to low staining intensity on the gels caused by the small amounts of muscle tissue available from each fish. Although four alleles have been found at this locus only two were identified in tested adult fish. Out of 106 individuals all were homozygous for allele 2 except for two fish heterozygous for this allele and allele 3. As with the 6 PGD locus the possibility of using this enzyme in inheritance studies appeared remote but IDH was stained for on gels run primarily to identify ADA polymorphisms.

(g) ADA

Polymorphism in adenosine deaminase was first described in red cell lysates of man (Spencer et. al., 1968). A single locus with two alleles producing single banded homozygotes and double banded heterozygotes was observed. More recently several rare alleles, including a 'null' allele have been described together with tissue specific secondary isozymes, presumed to be derived from the red cell ADA (Harris and Hopkinson, 1976).

In fish the first report of this enzyme was in the viviparous blenny Z. viviparus (Frydenberg and Simonsen, 1973); a single polymorphic locus with three alleles controlling single banded homozygotes and double banded heterozygotes was described

in heart muscle. A single locus was also reported in three species of Xiphophorus (Morizot, Wright and Siciliano, 1977) but no polymorphism was found. However Ward and Beardmore (1977) described one polymorphic locus, with six alleles, controlling ADA in the plaice. This locus was one of the five included by Beardmore and Ward (1977) in their previously mentioned discussion of heterozygosity levels in plaice.

This locus was only tested for in 1977 and 1978. Polymorphism was higher than at any other locus studied apart from PGM. Seven alleles have been identified in this study and six were found in the adult fish. Five samples of fish were collected and X^2 tests upon observed and expected phenotype distributions were possible for two of these; genetic equilibrium was indicated in both. Contingency tests on phenotype and allele distributions between all the samples showed homogeneity and a X^2 test upon overall observed and expected phenotype distributions indicated genetic equilibrium.

Therefore although data exist for only five samples no indication of any genetical differences between the represented geographical sources are apparent at this locus.

(h) PK

This enzyme was only stained for in 1977. The results were not easily resolved and many individuals were untyped. Although polymorphism was interpreted in two fish as a single locus controlling a dimeric molecule no attempts were made to use this enzyme in the genetical crosses. The numbers of fish typed were too low for any further discussion.

As mentioned at the start of the results sections not all of the enzymes listed in the Methods section were used in the inheritance studies. The enzymes that have been discussed in

detail here are only those for which tests were attempted in these adult fish.

The range of geographical origins of the adult fish, combined with low levels of heterozygosity for some of the enzymes, makes detailed analysis of the data difficult. However within these limitations the results from these fish suggest that genetical differences may exist at the G3PDH locus between spawning plaice stocks from the central and southern North Sea. No differences between samples were detected at three loci, MDH-A, PGM and ADA indicating homogeneity between these stocks with respect to these loci. At four other loci, GPI-A and B,6PGD and IDH no statistical analysis was possible.

CONVENTIONAL CROSSES

RESULTS

Offspring from over 200 crosses were tested during the five spawning seasons 1974-78. In each cross the numbers of offspring of each type were compared to the numbers expected assuming that the characters were inherited in a Mendelian manner and that their genetical control was as deduced from the study of the adults. The results show that for each system the enzyme variants were transmitted in accordance with the simple hypothesis that they were determined by codominant alleles, although occasional anomalies were observed. In all crosses between an assumed homozygote and a heterozygote the offspring segregated into two classes. Single phenotypes in both parents and offspring are therefore assumed to be homozygous for the allele in question. Some crosses were replicated. The results from such crosses will be pooled but any evidence of heterogeneity will be mentioned.

Frequently parents were polymorphic at more than one of the loci. Whenever possible crosses were set up using such fish either in attempts to study linkage or to increase the amount of data available without increasing husbandry.

Anomalies to the expected segregations fell into three categories:

(i) Segregation of offspring in compliance with a Mendelian ratio but not the ratio expected from the parental phenotypes. This occurred only twice in 1974 and could be explained on the basis of mistyping one parent or misreading a parental tag. The results otherwise complied with the expected Mendelian ratios and will not be further discussed.

(ii) Anomalies of segregation ratios which cannot be explained by mis-identification of parents.

(iii) The occurrence of unexpected individual genotypes, in some instances possessing an allele not present in either parent.

The examples of (ii) and (iii) will be reported as they occur when the results for each locus are presented.

(a) G3PDH

Table 21 lists the results of all the crosses tested. In this table and throughout the whole study the female parent is listed first. All the data except for one cross were obtained in 1978. As previously mentioned (see Adult plaice - Results) G3PDH does not give a strong reaction using small amounts of tissue and it was not until 1978, when an appreciable amount of data had been obtained at more easily identifiable loci, that extensive efforts could be made to rear fish to a size large enough for successful typing to take place. In one of the crosses only a single offspring survived. However in the remaining crosses and the pooled data the distribution of phenotypes does not deviate significantly from the distribution expected assuming Mendelian inheritance.

(b) MDH

These results refer only to the polymorphic locus MDH-A; throughout this study the MDH-B locus appeared to be monomorphic. The results of 30 crosses are given in Table 22. Successful typing of this enzyme is possible in larvae within a few days of hatching. All the crosses, except one, were large enough for statistical analysis and deviation from the expected segregation of offspring was never observed. Grouping the data for each type of cross into

Table 21 Conventional crosses - Segregation of offspring at the G3PDH locus

Parent genotypes	Year	Cross No.	Offspring genotypes				X^2	Probability
			2-2	1-2	1-3	2-3		
2-2 x 1-2	1978	17	46	47			0.01	0.05>0.90
		48	38	49			1.39	0.30>0.20
		64	2	2			.00	P >0.99
Total			86	98			0.78	0.50>0.30
2-3 x 2-3	1977	1	6			3	1.00	0.50>0.30
	1978	13	43			55	1.47	0.30>0.20
		41	1			2	0.33	0.70>0.50
		65	-			1		
Total			50			61	1.09	0.30>0.20
1-2 x 2-2	1978	19	3	1			1.00	0.50>0.30
		47	18	26			1.45	0.30>0.20
		49	24	21			0.20	0.70>0.50
		54	7	5			0.33	0.70>0.50
Total			52	53			0.01	0.95>0.90
1-2 x 1-3	1978	31	2	2	1	2	0.43	0.95>0.90
2-3 x 2-2	1978	67	2			5	1.29	0.30>0.20

Table 22 Conventional crosses - Segregation of offspring at the MDH-A locus

Parent genotypes	Year	Cross No.	Offspring genotypes			X^2	Probability
			2-2	3-3	2-3		
2-2 x 2-3	1975	DT6	58		55	0.08	0.80 > 0.70
	1976	3+11	98		98	0.00	P > .99
		4	73		69	0.11	0.80 > 0.70
		22	61		62	0.01	0.95 > 0.90
		26	33		24	1.42	0.30 > 0.20
	1978	2	46		62	2.37	0.20 > 0.10
		5	18		19	0.03	0.90 > 0.80
		13	53		45	0.65	0.50 > 0.30
		17	51		42	0.87	0.50 > 0.30
		31	2		5	1.29	0.30 > 0.20
		41	2		1	0.33	0.70 > 0.50
		65	1				
Total			496		482	0.20	0.70 > 0.50
2-3 x 2-2	1975	15	16		11	0.93	0.50 > 0.30
		16+26	46		63	2.65	0.20 > 0.10
		18	5		7	0.33	0.70 > 0.50
		19+37	106		98	0.31	0.70 > 0.50
	1976	15	52		38	2.18	0.20 > 0.10
		19	40		36	0.21	0.70 > 0.50
		25	49		53	0.16	0.70 > 0.50
		28	4		3	0.14	0.80 > 0.70
		29	116		107	0.36	0.70 > 0.50
Total			434		416	0.38	0.70 > 0.70
2-3 x 2-3	1975	DT4	11	12	29	0.73	0.70 > 0.50
		25	13	14	23	0.36	0.90 > 0.80
		27	2	4	11	1.94	0.50 > 0.30
		34	3	7	6	3.00	0.30 > 0.20
	1976	2	12	15	19	1.78	0.50 > 0.30
	1977	1	5	-	4	5.67	0.10 > 0.05
Total			46	52	92	0.57	0.50 > 0.30

years maintained this agreement, as did overall totals. Contingency tests between yearly distribution for the 2-2 x 2-3 and 2-3 x 2-2 crosses showed no differences (X^2 = 0.16, D.F. = 2, $0.95 > P > 0.90$ and X^2 = 0.88, D.F. = 1, $0.50 > P > 0.30$ respectively).

Homozygous 2-2 x 2-2 crosses were tested in 1976 (1 mating) and 1978 (7 matings). A total of 317 offspring were examined: all were 2-2 homozygotes.

(c) PGM

Fifty three crosses involving four alleles were tested at this locus. Forty of these crosses included alleles 3 and 4 and the thirty-five of these containing at least one heterozygous parent, are listed in Table 23. The remaining five crosses were between homozygous parents. The other thirteen matings were between an assortment of phenotypes, see Table 24.

In most of the crosses, segregation of the offspring was consistent with expectation. However there were exceptions, where anomalies of segregation ratios cannot be explained by misidentification of parents. These most frequently occurred in the 3-4 x 3-4 crosses where four of the nine crosses examined gave irregular segregation ratios. Two of these, 1974 cross number 4 and 1978 cross number 71 had an excess of 3-3 genotypes. Another, 1975 cross number DT8, had an excess of 4-4 genotypes whilst the remaining cross, 1974 number 3, had an excess of 3-3 and a deficiency of 4-4 genotypes.

The same offspring from three of these four crosses were also tested for segregation at another polymorphic locus where the results conformed to Mendelian ratios.

Table 23 Conventional crosses - Segregation of offspring at the PGM locus. The results of all the matings involving heterozygotes carried out between 3-3, 4-4 and 3-4 phenotypes.

Parent genotypes	Year	Cross No.	Offspring genotypes				X^2	Probability
			3-3	4-4	2-3	3-4		
3-3 x 3-4	1976	19	41		1	36	0.33	0.70 > 0.50
4-4 x 3-4	1974	6		44		42	0.04	0.90 > 0.80
		10		51		50	0.01	0.95 > 0.90
	1976	21		131		109	2.02	0.20 > 0.10
		23		143		139	0.06	0.90 > 0.80
		28		60		76	1.88	0.20 > 0.10
	1978	2		52		48	0.16	0.70 > 0.50
		3		101		93	0.33	0.70 > 0.50
		13		55		51	0.15	0.70 > 0.50
		48		12		8	0.80	0.50 > 0.30
Total				649		616	0.86	0.50 > 0.30
3-4 x 3-3	1976	2+6+12	239			261	0.97	0.50 > 0.30
		24	78			81	0.06	0.90 > 0.80
Total			315			342	1.11	0.30 > 0.20
3-4 x 4-4	1974	2		22		13	2.31	0.20 > 0.10
	1975	DT4	1	22		29	0.96	0.50 > 0.30
		DT6		44		69	5.53	0.02 > 0.01
		15		10		13	0.39	0.70 > 0.50
		18		2		10	5.33	0.05 > 0.02
		36		29		32	0.15	0.80 > 0.70
	1976	4		49		53	0.16	0.70 > 0.50
		15		50		49	0.01	0.95 > 0.90
		26	1	43		56	1.70	0.20 > 0.10
		29		117		136	1.43	0.30 > 0.20
		40		12		5	2.88	0.10 > 0.05
		43		51		60	0.73	0.50 > 0.30
Total			2	451		525	5.91	0.02 > 0.01
3-4 x 3-4	1974	1	7	8		16	0.09	0.98 > 0.95
		3	69	8	1	48	65.75	0.001 > P
		4	15	5		11	9.06	0.02 > 0.01
		5	10	5		15	1.67	0.50 > 0.30
		11	2	6		10	2.00	0.50 > 0.30
	1975	DT8	21	45		56	10.26	0.01 > 0.001
	1976	20	28	24		48	0.48	0.80 > 0.70
		27	26	24		48	0.12	0.80 > 0.70
	1978	71	22	14		22	5.59	0.02 > 0.01
Total			200	139	1	274	19.00	0.001 > P

Table 24 Conventional crosses - Segregation of offspring at the PGM locus. The results of all the other matings involving heterozygotes carried out apart from those listed in Table 23.

Parent genotypes	Year	Cross No.	Offspring genotypes							X^2	Probability
			3-3	4-4	1-3	1-4	2-3	2-4	3-4		
4-4 x 1-4	1975	21		3		1				1.00	0.50>0.30
1-3 x 3-3	1976	3+5+11	240		203	Plus 1	individual	1-2		3.09	0.10>0.05
		14	46		56					0.98	0.50>0.30
Total			286		259					1.34	0.30>0.20
1-3 x 4-4	1976	22				41			60	3.57	0.10>0.05
1-4 x 3-4	1978	4		2	-	5			-		
		5		51	49	51			63	2.30	0.70>0.50
		10		1	-	-			2		
Total				54	49	56			65	2.39	0.50>0.30
3-4 x 1-4	1975	32		31	18	28			18	5.76	0.20>0.10
3-4 x 2-4	1974	8		15			14	9	20	4.21	0.20>0.10
3-4 x 2-3	1974	7	14	1			3	7	14	11.10	0.02>0.01
		9	27				19	18	26	2.89	0.50>0.30
Total			41	1			22	25	40	9.12	0.05>0.02

Both 1974 cross 3 and 1975 cross DT8 involved heterozygotes at the GPI-B locus (parents 6-6 x 4-6 offspring 6-6 62, 4-6 66; $X^2 = 0.12$; $0.80 > P > 0.70$ and parents 4-6 x 6-6 offspring 6-6 59, 4-6 63; $X^2 = 0.13$; $0.80 > P > 0.70$ respectively) whilst the third cross, 1974 number 4, had one parent heterozygous at the GPI-A locus (parents 1-2 x 2-2 offspring 2-2 19, 1-2 16; $X^2 = 0.28$; $0.70 > P > 0.50$).

The overall distribution of offspring from all the nine 3-4 x 3-4 crosses indicates an excess of 3-3 homozygotes but this is mainly caused by the large irregularity in distribution in 1974 cross 3. Removal of this cross from the total gives agreement with expected distribution (3-3 131, 4-4 131, 3-4 244; $X^2 = 2.66$; $0.30 > P > 0.20$).

Other irregularities occurred in two 3-4 x 4-4 crosses in 1975, numbers DT6 and 18, where significant excesses of heterozygote offspring 3-4 were found. These offspring were also tested at the MDH-A locus where segregation was as expected (cross DT 6 parents 2-2 x 2-3 offspring 2-2 58, 2-3 55; $X^2 = 0.08$; $0.80 > P > 0.70$; cross 18 parents 2-3 x 2-2 offspring 2-2 5, 2-3 7; $X^2 = 0.33$; $0.70 > P > 0.50$). Although offspring from the remainder of the 3-4 x 4-4 crosses did not deviate from expected segregation ratios the overall total of offspring from the 12 crosses indicated a significant excess of 3-4 heterozygotes. When the data for each year are analysed the 1975 offspring nunbers deviate significantly from the expected 1 : 1 ratios (3-3 1, 4-4 107, 3-4 153; $X^2 = 8.11$; $0.01 > P > 0.001$), whilst those of 1976 do not (4-4 322, 3-4 359; $X^2 = 2.01$; $0.20 > P > 0.10$), although more 3-4 offspring than 4-4 offspring were observed.

The final segregation ratio anomaly occurred in one of the two 3-4 x 2-3 crosses tested in 1974 cross number 7. This cross was significantly deficient in allele 2, but segregation for the same offspring when tested at the GPI-B locus was as expected (parents 4-6 x 6-6 offspring 6-6 23, 4-6 18; $X^2 = 0.61$; $0.50 > P > 0.30$). The overall totals for the offspring of both 3-4 x 2-3 crosses gave a significant deficit of allele 2.

Individual anomalies were also found in six crosses as follows:

(i) a 2-3 phenotype in a 3-3 x 3-4 cross in 1976 (cross number 19)
(ii) a 3 phenotype in a 3-4 x 4-4 cross in 1975 (cross number DT4)
(iii) a 3 phenotype in a 3-4 x 4-4 cross in 1976 (cross number 26)
(iv) a 2-3 phenotype in a 3-4 x 3-4 cross in 1974 (cross number 3)
(v) a 1-2 phenotype in a 1-3 x 3-3 cross in 1976 (cross number 5)
(vi) a 4 phenotype in a 3-4 x 2-3 cross in 1974 (cross number 7)

The possibility of contamination from other crosses causing the occurrence of both the unexpected segregation ratios and the individual anomalies may be eliminated in the majority of cases.

Considering the segregation ratios first, Table 25 gives full genotypic details for each of the seven relevant crosses. In all the crosses the offspring were tested for all the loci listed, and apart from the PGM locus the results were always as expected. In four of the crosses no contamination could have taken place to cause the abnormal PGM segregation without affecting the offspring at another locus because no such suitable crosses were made concurrently. In the remaining three examples, 1974 number 7 and 1975 numbers DT6 and 18, there was in each year another cross whose addition could have caused the PGM irregularities and yet

Table 25 Full details of the six crosses producing unexpected offspring segregation at the PGM locus, in chronological order.

Cross	Parent genotypes	Offspring genotypes										Comments
		2-2	3-3	4-4	6-6	1-2	1-3	2-3	2-4	3-4	4-4	
1974, no 3												
PGM	3-4 x 3-4		69	8				1		48		No possible contaminating cross made
GPI-A	2-2 x 2-2	128										
GPI-B	6-6 x 4-6				62						66	
1974, no 4												
PGM	3-4 x 3-4		15	5						11		No possible contaminating cross made
GPI-A	1-2 x 2-2	19				16						
GPI-B	6-6 x 6-6				35							
1974, no 7												
PGM	3-4 x 2-3		14	1				3	7	14		Only possible contaminant 1974 cross 3: PGM 3-4 x 3-4 GPI-A 2-2 x 2-2 GPI-B 6-6 x 4-6
GPI-A	2-2 x 2-2	41										
GPI-B	4-6 x 6-6				23						18	
1975, no DT6												
PGM	3-4 x 4-4			44						69		Only possible contaminant 1975 cross 26: PGM 3-3 x 4-4 MDH-A 2-3 x 2-2
MDH-A	2-2 x 2-3	58						55				
1975, no DT8												
PGM	3-4 x 3-4		21	45						56		No possible contaminating cross made
GPI-A	2-2 x 2-2	122										
GPI-B	4-6 x 6-6				59						63	
1975, no 18												
PGM	3-4 x 4-4			2						10		Only possible contaminant 1975 cross 26, already detailed above.
MDH-A	2-3 x 2-2	5						7				
1978, no 71												
PGM	3-4 x 3-4		22	14						22		No possible contaminating cross made
GPI-A	2-2 x 2-2	58										
GPI-B	6-6 x 6-6				58							

still have fitted with the results at the other loci tested. However, in view of the precautions taken and the abnormalities in the other four crosses, such physical mixing seems an unlikely explanation of the anomalies; the possibility of linked deleterious genes arises (see Discussion page 97).

Similarly the occurrence of the individual anomalies in certain of the broods is unlikely to have arisen by contamination. The presence of a 1-2 phenotype and a 2-3 phenotype in offspring from the 1976 crosses, numbers 5 and 19 respectively, could obviously not have been due to contamination since no phenotype involving allele 2 was present in the parent fish at that time. The simplest interpretation is that all these single abnormalities represent mutations. The PGM 3 phenotypes found in 1975 cross DT4 and 1976 cross 6 may be mutations from allele 2 to either allele 3 or a null allele. Similarly the PGM 4 phenotype found in 1974 cross 7 may be a mutation from either allele 2 or allele 3 to either allele 4 or a null allele. However as no evidence of a null allele has been found at this locus throughout this study it seems more likely that the mutations are from allele 2 to allele 3 and from either allele 2 or allele 3 to allele 4.

The offspring from five crosses between parents homozygous at the PGM locus but heterozygous at another locus were tested; two 4-4 x 4-4 matings and three 3-3 x 4-4 matings produced respectively 189 4-4 genotypes and 302 3-4 genotypes.

Further implications of the anomalies observed will be taken up later in the results and the discussion.

(d) GPI

The two loci controlling different forms of this enzyme will be dealt with separately.

GPI-A

Table 26 contains details of all the 13 crosses involving heterozygotes made at this locus. The four 2-2 x 2-3 crosses and the only 1-2 x 2-2 cross all gave offspring ratios in accordance with expectation. In five of the six 2-2 x 1-2 crosses made in 1976 a deficiency of heterozygous 1-2 offspring was found. Of the two 2-2 x 1-2 crosses tested in 1974 the offspring from one did not deviate from the expected ratio whilst in the other a deficiency of heterozygotes was present although the number of offspring in this cross were very low. A similar deficiency was present in the overall totals from all eight crosses.

Five of the anomalous crosses were also tested at another heterozygotic locus where segregation was always as expected. Details are given in Table 27.

The possibility of contamination causing the GPI-A 1-2 heterozygote deficiencies can be eliminated by the same procedure as used for the PGM anomalies. Table 28 gives details of all the loci examined in each of the six crosses and the parental genotypes. In every instance potential contamination would have to come from a GPI-A 2-2 x 2-2 cross, matching the respective parents of the original GPI-A 2-2 x 1-2 cross at the other loci. For crosses 1974 number 11 and 1976 numbers 25 and 45 no such possibility existed and for crosses 21, 23 and 43 from 1976 only two possible sources of contamination existed. The chances of contamination from these are remote, considering the precautions taken, the different dates of matings and the number of other crosses made concurrently.

Table 26 Conventional crosses - Segregation of offspring at the GPI-A locus.

Parent genotypes	Year	Cross no.	Offspring genotypes			X^2	Probability
			2-2	1-2	2-3		
2-2 x 1-2	1974	6	48	38		1.16	0.30 > 0.20
		11	14	3		7.12	0.01 > 0.001
	1976	21	148	92		13.07	0.001 > P
		23	131	101		3.88	0.05 > 0.02
		25	136	102		4.86	0.05 > 0.02
		29	141	112		3.32	0.10 > 0.05
		43	69	42		6.57	0.02 > 0.01
		45	76	32		17.93	0.001 > P
Total			763	522		45.20	0.001 > P
2-2 x 2-3	1975	36	32		28	0.27	0.70 > 0.50
	1976	22	24		27	0.18	0.70 > 0.50
		26	58		42	2.56	0.20 > 0.10
	1978	3	95		99	0.08	0.80 > 0.70
Total			209	196		0.42	0.70 > 0.50
1-2 x 2-2	1974	4	19	16		0.26	0.70 > 0.50

Table 27. Details of offspring segregation at other loci for five of the crosses giving anomalous segregations at the GPI-A locus.

	Parents		Offspring		X^2	Probability
1974 Cross 11 : PGM	3-4 x 3-4		3-3	2	2.00	0.50 > 0.30
			4-4	6		
			3-4	10		
GPI-B	4-6 x 6-7		6-6	6	4.00	0.50 > 0.30
			4-6	2		
			4-7	2		
			6-7	2		
Cross 21 : PGM	4-4 x 3-4		4-4	131	2.02	0.20 > 0.10
			3-4	109		
Cross 23 : PGM	4-4 x 3-4		4-4	143	0.06	0.90 > 0.80
			3-4	139		
Cross 25 : MDH-A	2-3 x 2-2		2-2	49	0.16	0.70 > 0.50
			2-3	53		
Cross 43 : PGM	3-4 x 4-4		4-4	51	0.73	0.50 > 0.30
			3-4	60		

Table 28 Full details of the six GPI-A 2-2 x 1-2 crosses producing unexpected offspring segregation, in chronological order.

Cross	Parent genotypes	Offspring genotypes										Comments
		2-2	3-3	4-4	6-6	1-2	2-3	3-4	4-6	4-7	6-7	
1974, no 11												
PGM	3-4 x 3-4		2	6				10				No possible contaminating cross made
GPI-A	2-2 x 1-2	14				3						
GPI-B	4-6 x 6-7				6				2	2	2	
1976 no. 21												
PGM	4-4 x 3-4			131				109				Only possible contaminants for crosses 21 and 23
GPI-A	2-2 x 1-2	148				92						
GPI-B	6-6 x 6-6				239						1	1976 cross 4: PGM 3-4 x 4-4
												GPI-A 2-2 x 2-2
												GPI-B 6-6 x 6-6
no. 23												
PGM	4-4 x 3-4			143				139				1976 cross 28: PGM 4-4 x 3-4
GPI-A	2-2 x 1-2	131				101						GPI-A 2-2 x 2-2
GPI-B	6-6 x 6-6				282							GPI-B 6-6 x 6-6
no. 25												
PGM	3-3 x 4-4							238				No possible contaminating cross made
GPI-A	2-2 x 1-2	136				102						
GPI-B	6-6 x 6-6				238							
MDH-A	2-3 x 2-2	49					53					
no. 43												
PGM	3-4 x 4-4			51				60				Only possible contaminants 1976 crosses 4 and 28 see above
GPI-A	2-2 x 1-2	69				42						
GPI-B	6-6 x 6-6				111							
no. 45												
PGM	4-4 x 4-4			108								No possible contaminating cross made
GPI-A	2-2 x 1-2	76				32						
GPI-B	6-6 x 6-6				108							

Purdom et. al. (1976) suggested that the fitness of the 1-2 offspring may be lower than that of the 2-2 offspring and in 1976 an experiment was carried out to test this hypothesis. Larvae from four of the 1976 crosses, numbers 21, 23, 25 and 29 were reared to metamorphosis and 20 days after hatching larvae from each cross were divided into groups and reared at three available different temperatures, 7, 11 and 15°C. Samples of about 50 individuals were removed at intervals and tested, see Table 29. Although mortalities occurred throughout the rearing procedure it was not possible to accurately type the dead larvae due to the rapid decomposition of the corpses; consequently genetic composition of the survivors only was examined. In an attempt to determine the effect of the range of temperatures and range of sampling dates a heterogeneity chisquare of phenotype distribution throughout all the samples, irrespective of cross, age and rearing temperature gave no evidence of any irregularity in distribution (X^2 = 34.22, D.F. = 23, $0.10 > P > 0.05$).

No evidence of any irregularity in distribution within the samples was apparent when similar tests were applied to all the larvae reared at 7°C until 20 days old (X^2 = 13.55, D.F. = 10, $0.20 > P > 0.10$), the fish reared at 7°C (X^2 = 7.48, D.F. = 4, $0.20 > P > 0.10$) and those reared at 11°C (X^2 = 9.89, D.F. = 6, $0.20 > P > 0.10$). Only one sample of fish was reared at 15°C. A similar comparison between the totals of these four groups showed no significant differences in the distribution of genotypes (X^2 = 2.17, D.F. = 3, $0.70 > P > 0.50$).

Although heavy mortalities occurred at metamorphosis no differences in genotype distribution between larvae and fish were found (X^2 = 0.17, D.F. = 1, $0.70 > P > 0.50$).

Table 29. The offspring from the six 2-2 x 1-2 crosses made in 1976 are divided by age.

Cross no.	Age (days)	Offspring genotypes		X^2	Probability	% age hets.	Rearing conditions (all larvae reared at 7°C until day 20)
		2-2	1-2				
21	10	25	27	0.08	0.80 > 0.70	51.92	Larvae at 7°C.
	13	33	23	1.79	0.20 > 0.10	41.07	Larvae at 7°C
	43	31	15	5.57	0.02 > 0.01	32.61	Fish at 11°C
	70	38	15	9.93	0.01 > 0.001	28.30	Fish at 7°C
	79	21	12	2.45	0.20 > 0.01	36.36	Fish at 7°C
23	11	25	26	0.02	0.80 > 0.70	50.98	Larvae at 7°C
	45	31	19	2.88	0.10 > 0.05	38.00	Fish at 11°C
	72	30	26	0.29	0.70 > 0.50	46.43	Fish at 11°C
	72	21	10	3.90	0.05 > 0.02	32.26	Fish at 7°C
	72	24	20	0.36	0.70 > 0.50	45.45	Fish at 15°C
25	17	26	24	0.08	0.80 > 0.70	48.00	Larvae at 7°C
	29	35	18	5.45	0.02 > 0.01	33.96	Larvae at 7°C
	45	22	28	0.72	0.50 > 0.30	56.00	Fish at 11°C
	77	32	20	2.77	0.10 > 0.05	38.46	Fish at 11°C
	81	21	12	2.45	0.20 > 0.10	36.36	Fish at 7°C
29	23	32	25	0.86	0.50 > 0.30	43.86	Larvae at 7°C
	24	36	22	3.38	0.10 > 0.05	37.93	Larvae at 7°C
	51	24	27	0.18	0.70 > 0.50	52.94	Fish at 11°C
	66	35	19	4.74	0.05 > 0.02	35.19	Fish at 11°C
	82	14	19	0.76	0.50 > 0.30	57.58	Fish at 7°C
43	11	33	23	1.79	0.20 > 0.10	41.07	Larvae at 7°C
	15	36	19	5.25	0.05 > 0.02	34.55	Larvae at 7°C
45	17	38	18	7.14	0.01 > 0.001	32.14	Larvae at 7°C
	18	38	14	11.08	0.001 > P	26.92	Larvae at 7°C

Since no apparent differences between samples were caused by the variety of temperatures and different individual crosses any effect of age on phenotype distribution was examined by a linear regression. A plot of all the samples showed no significant deviation from zero, see Figure 3.

Therefore although the significant deficiencies in heterozygote offspring in these crosses suggest that the 1-2 genotype is less fit than the 2-2 genotype under the experimental conditions (X^2 = 40.95, D.F. = 1, 0.001>P) there is no evidence that this has anything to do with temperature or age over the period covered. Any inviability must be expressed earlier in the life history.

Thirty-two 2-2 x 2-2 crosses were tested, six in 1974, three in 1975, fifteen in 1976 and eight in 1978, totalling 3,030 offspring. All were phenotype 2 homozygotes.

GPI-B

Forty-five crosses were examined at this locus between 1974 and 1978. The majority of these were from homozygous 6-6 x 6-6 parents whose offspring were also being tested for segregation at other polymorphic loci. Details of the remaining 20 crosses are given in Table 30. Unexpected segregation ratios were found in 5 of the crosses as follows:

(i) The offspring of a pair of parents of genotype 6-6 x 4-6 (1976, crosses number 3 + 5 + 11) gave an excess of 6-6 offspring; this excess was due to only one of the three matings, number 5 (offspring 6-6 85, 4-6 60; X^2 = 4.31; 0.05>P>0.02). This mating gave an expected segregation at the PGM locus apart from a single anomaly (parents 1-3 x 3-3 offspring 3-3 72, 1-3 72, 1-2 1; X^2 = 0.01; 0.95>P>0.90), as did the overall distribution for the

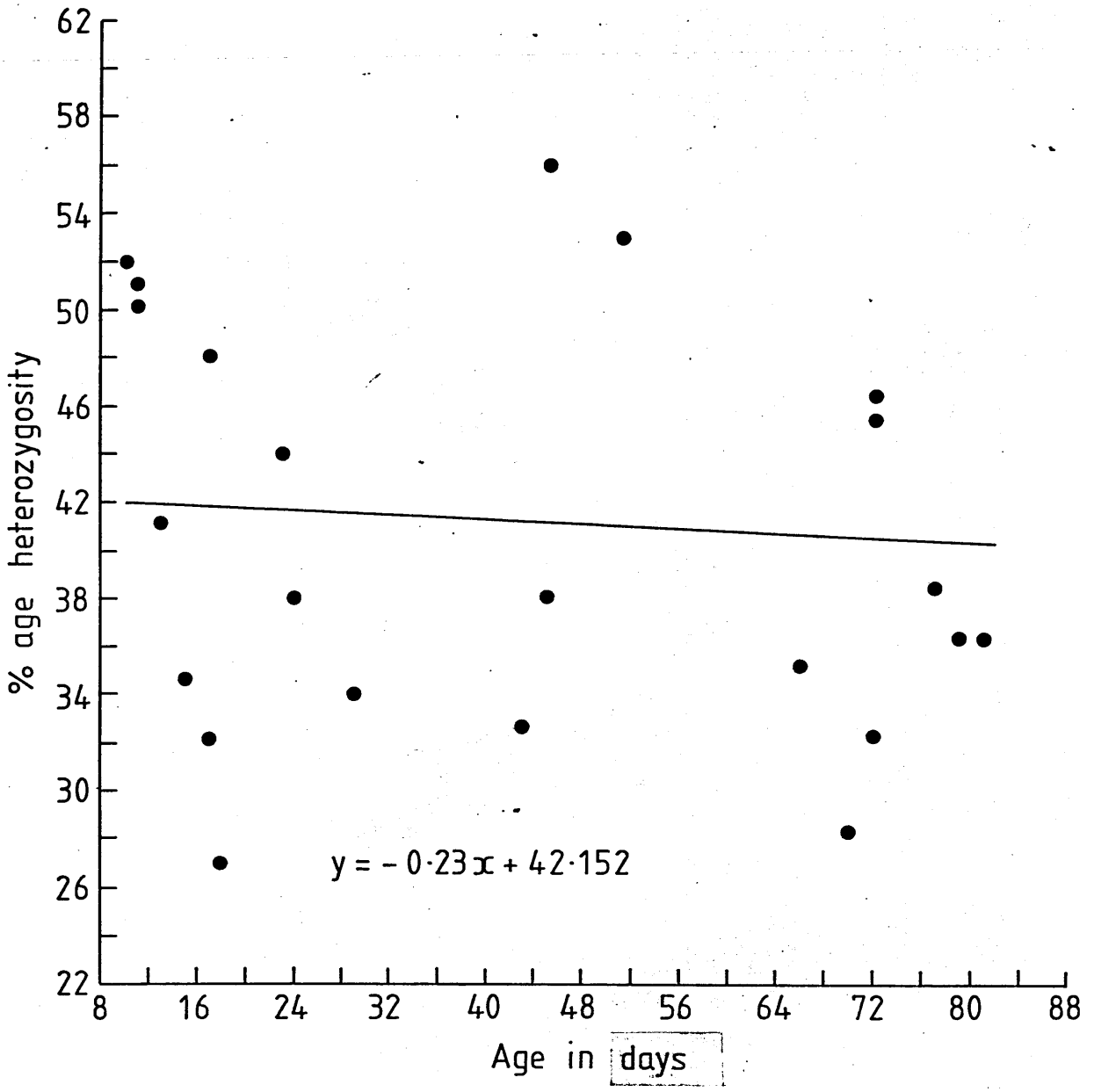

Fig 3. Heterozygosity v age

Table 30 Conventional crosses - Segregation of offspring at the GPI-B locus.

Parent genotypes	Year	Cross No.	Offspring genotypes								X^2	Probability
			4-4	6-6	2-6	4-5a	4-6	4-7	5a-6	6-7		
6-6 x 2-6	1976	20		50	50						0.00	P > 0.99
		27		38	34						0.22	0.70 > 0.50
Total				88	84						0.09	0.80 > 0.70
6-6 x 4-6	1974	3		62			66				0.12	0.80 > 0.70
	1976	3+5+11		251			196				6.77	0.01 > 0.001
		40		9			8				0.06	0.90 > 0.80
	1978	6		46			63				2.65	0.20 > 0.10
Total				368			333				1.75	0.20 > 0.10
6-6 x 6-7	1974	6		56						30	7.86	0.01 > 0.001
4-6 x 6-6	1974	7		23			18				0.61	0.50 > 0.30
	1975	DT8		59			63				0.13	0.80 > 0.70
		21		-			4				3.00	0.10 > 0.05
Total				82			85				0.05	0.90 > 0.80
4-6 x 4-6	1974	5	4	10			7				5.76	0.02 > 0.01
4-6 x 5a-6	1976	24		36		26	53		44		0.98	0.05 > 0.02
4-6 x 6-7	1974	11		6			2	2		2	4.00	0.50 > 0.30
6-7 x 6-6	1976	15		102						96	0.18	0.70 > 0.50
		29		130						123	0.19	0.70 > 0.50
Total				232						219	0.37	0.70 > 0.50
6-7 x 4-6	1976	2+6+12		141			117	99		132	8.28	0.05 > 0.02

three matings made between these individual fish (offspring 3-3 240, 1-3 203, 1-2 1; $X^2 = 3.09$; $0.10 > P > 0.05$). This single unexpected ratio did not upset the overall segregation from pooled data from the four sets of genotype 6-6 x 4-6 parents.

(ii) The only 6-6 x 6-7 cross tested (1974, number 6) had an excess of 6-6 offspring; this cross gave expected segregations at both the PGM locus (parents 4-4 x 3-4 offspring 4-4 44, 3-3 42; $X^2=0.05$; $0.90 > P > 0.80$) and the GPI-A locus (parents 2-2 x 1-2 offspring 2-2 48, 1-2 38; $X^2 = 1.16$; $0.30 > P > 0.20$).

(iii) The larvae from the only 4-6 x 4-6 cross tested (1974, number 5) gave an anomalous result, again an excess of 6-6 genotypes but the number of offspring tested was low. These larvae when tested at the PGM locus gave an expected ratio (parents 3-4 x 3-4 offspring 3-3 4, 4-4 4, 3-4 11; $X^2 = 0.47$; $0.50 > P > 0.30$).

(iv) The progeny of the 4-6 x 5^a-6 cross tested in 1976 (number 24) were low in 4-5^a genotypes but again when tested at the PGM locus an expected segregation was found (parents 3-4 x 3-3 offspring 3-3 78, 3-4 81; $X^2 = 0.06$; $0.90 > P > 0.80$).

(v) Offspring from the 6-7 x 4-6 cross (1976, numbers 2 + 6 + 12) showed an excess of allele 6, although when treated individually only one of the three matings between these two parents, number 12, showed this significantly (offspring 6-6 52, 4-6 42, 4-7 21, 6-7 37; $X^2 = 13.21$; $0.01 > P > 0.001$). No anomaly was observed in this mating at the PGM locus (parents 3-4 x 3-3 offspring 3-3 80, 3-4 76; $X^2 = 0.10$; $0.80 > P > 0.70$), nor in the overall PGM segregation for the three matings made between these parents (offspring 3-3 239, 3-4 261; $X^2 = 0.97$; $0.50 > P > 0.30$).

Twenty-five 6-6 x 6-6 crosses were tested, 4 in 1974, 2 in 1975, 11 in 1976 and 8 in 1978, giving a total of 2,581 offspring. Of these 2,580 were homozygous 6-6 genotypes as expected but in 1976, cross 21, a single heterozygote 6-7 genotype fish was found in a brood of 240 individuals. Once again the possibility that the anomaly arose from contamination was remote and the most likely explanation of the single anomaly is a mutation from allele 6 to allele 7.

All these anomalies will be given further consideration in a later results section and the discussion.

(e) 6PGD

All the crosses tested at this locus were between homozygous 2-2 x 2-2 parents. Thirteen crosses were examined in 1976 and one in 1977 giving a total of 1,546 offspring. All were of the homozygous 2-2 genotype.

(f) IDH

Only one 2-2 x 2-2 cross was tested at this locus in 1977 (number 15). Fifteen offspring were typed - all were 2-2 genotypes.

(g) ADA

Offspring from five crosses were tested in 1977 and 1978. Table 31 gives details. The numbers of progeny were not large in any of the crosses and in two cases were very small. However in all five the expected segregation ratios were observed. The reason for testing only five crosses at this locus and the cause of the low number of individuals in each cross was that this enzyme can be typed reliably only in metamorphosed fish.

Table 31 Conventional crosses - Segregation of offspring at the ADA locus

Parent genotypes	Year	Cross No.	Offspring genotypes						X^2	Probability
			2-2	4-4	6-6	2-4	2-6	4-6		
4-4 x 2-4	1977	15		8		10			0.22	0.70 > 0.50
	1978	5		20		17			0.24	0.70 > 0.50
Total				28		27			0.02	0.90 > 0.80
2-4 x 4-4	1977	12		26		31			0.44	0.70 > 0.50
2-6 x 2-6	1978	2	1		3		5			
2-6 x 4-6	1977	2			3	3	5	3		

DISCUSSION

As detailed in the previous section polymorphism has been reported at the enzyme loci studied in this project in many different fish species but the genetical control of such variation has usually been assumed by analogy to other species.

Apart from salmonid experiments, which, where relevant, will be discussed later when the results obtained in this study for each enzyme are considered, family data are limited.

Considerable studies of enzyme inheritance have been made in interspecific hybrids of the family Centrarchidae (Champion and Whitt, 1976; Wheat et al., 1971, 1972, 1973, 1974; Whitt, Childers, Tranquilli and Champion, 1973b; Whitt et al., 1973a, 1976) and the family Poecilidea (Morizot et al., 1977; Scholl and Anders, 1973; Siciliano and Wright, 1973) but these rely upon inter not intraspecific differences to determine linkage and allelic expression; they will not be discussed here.

In marine fish Hjorth (1971) studied mother/offspring relationships at the PGM loci in broods of the viviparous blenny, Zoarces viviparus, but these were not controlled matings as the females were fertilized before capture.

The only true breeding studies in marine fish have been carried out in plaice (de Ligny, 1970; Dando, 1974; Purdom et al., 1976; Ward and Beardmore, 1977) and in flounder, Platychthys flesus (Dando, 1974). De Ligny (1970) described transferrin inheritance but interpretation was complicated by an ontogenic change in the number of transferrin bands produced by individuals. Only a few crosses were made and the numbers of offspring were small. Apart from this work, and the flounder

data presented by Dando (1974), all the other matings and husbandry have been carried out in the Lowestoft laboratory. Purdom et al. (1976) included the 1974 and 1975 data discussed here together with some earlier work carried out by Dando. These previously published data will be discussed when the relevant enzymes are considered in more detail.

Apart from wishing to establish the genetical control of the enzyme polymorphisms identified in the adult fish it was essential to examine the segregation in offspring from normal matings before studying the inheritance of polymorphism in gynogenetic diploid and induced triploid larvae.

As in the results section each enzyme will be considered individually.

(a) G3PDH

Throughout the crosses made for this locus the segregations of offspring never deviated significantly from those expected, assuming Mendelian control. No evidence of any differential fitness was found amongst genotypes, though unfortunately the numbers of individuals in some of the crosses were very low due to the heavy mortalities incurred in rearing flatfish larvae through to metamorphosis, a practice made necessary by the weak reaction given by this enzyme in small amounts of tissue. Purdom et al. (1976) presented some earlier crosses made at this locus; whilst five of the seven matings produced expected offspring segregation ratios excesses of homozygotes were found in the other two. However at this time the combination of the weak reaction of this enzyme, the use of frozen material and the electrophoretic conditions were presenting problems in

identification and the proportion of untyped individuals was high. The weaker staining heterozygotes may have been missed; the use of fresh material and a different buffer system in this study made identification much simpler.

Mendelian inheritance for this enzyme has also been demonstrated in the pink salmon, Oncorhynchus gorbuscha (Aspinwall, 1973), the lake whitefish, Coregonus clupeaformis (Clayton et al., 1973a) and the rainbow trout, Salmo gairdneri (Utter et. al., 1973b).

(b) MDH

Matings were made to study the MDH-A form of this enzyme in four separate years. Assuming Mendelian inheritance no significant deviations from the expected distribution of larval genotypes occurred throughout. The 1975 data presented here were included in Purdom et al. (1976). Ward and Beardmore (1977) included results of two matings for this locus; although the numbers of offspring were small they supported the findings of this study. Breeding data have established the genetical control of MDH polymorphism in four other species: the walleye, Stizostedion vitreum vitreum (Clayton et. al., 1971), the rainbow trout, S. gairdneri (Bailey et. al., 1970; Utter et al., 1973b; Allendorf et. al., 1975; Clayton, Tretiak, Billeck and Ihrsen, 1975), the king or chinook salmon, Oncorhynchus tshawytscha (Bailey et al., 1970; Kristiansson and McIntyre, 1976) and the pink salmon, O. gorbuscha (Aspinwall, 1974).

(c) PGM

More matings were made at this locus than at any of the others examined in this study. The reasons were simple. Firstly more

polymorphism was found at this locus than any of the others, hence the incidence of sexually mature and genetically suitable parent fish was greater. Secondly, the staining reaction of this enzyme on electropherograms was strong enough to allow the testing and consistent typing of yolksac larvae. Therefore the problems of the availability of suitable parent fish and the heavy mortalities in larval rearing were not constraints in experiments carried out at this locus.

Of the 44 crosses involving at least one heterozygous parent, and producing sufficient numbers of offspring to allow statistical analysis, all except seven gave segregation ratios consistent with Mendelian inheritance. Full details of the seven exceptions have been given previously (Table 25). In all these anomalies the identity of the parents was unique to that particular mating. Although all the females involved were of phenotype 3-4, 21 other crosses with such females gave expected results. Of the fourteen adults contributing to the seven anomalous crosses only one, the phenotype 4, male from 1975 cross DT6, phenotypes 3-4 x 4, was involved in another mating, 1975 cross DT4, again with a phenotype 3-4 female, but in this instance the results were not anomalous. As already explained in detail in the results physical mixing between broods could not account for four of the seven anomalies and, in view of the precautions taken, seems improbable in the remaining three.

Irregular segregations may arise by chance when large numbers of crosses are being tested. An alternative explanation is the presence of linked deleterious genes. This possibility cannot be established without either further crosses involving

the parent fish or rearing the offspring to sexual maturity and carrying out another series of crosses with them. Neither of these experiments could be carried out; the former because, apart from a few exceptions, adult fish were not kept from year to year, and the latter because of the restrictions of facilities and the time, up to three years, taken for plaice to reach sexual maturity.

Besides including four of the anomalies discussed here Purdom et al. (1976) also reported four similar irregularities discovered in experiments carried out before the commencement of this study in 1974; again no obvious explanation was available. Ward and Beardmore (1977) included data for four crosses at this locus; the results were not anomalous but in two of the crosses the numbers of offspring were too small.

The individual anomalies observed in some crosses will be discussed separately.

The Mendelian inheritance of PGM polymorphism has previously been demonstrated in the viviparous blenny, Z. viviparus (Hjorth, 1971), the sockeye salmon, Oncorhynchus nerka (Utter et al., 1973b) and the king or chinook salmon, O. tshawytscha (Kristiansson and McIntyre, 1976).

(d) GPI

The two loci coding for this enzyme will again be treated separately.

(i) GPI-A: Only thirteen crosses involving heterozygotes were tested at this locus due to the low level of polymorphism observed in the adult fish. Identifying this enzyme in yolksac larvae was not a problem, hence the large number of homozygous

2-2 x 2-2 crosses tested; all these matings were heterozygous for another locus. In the majority of crosses the segregation of offspring phenotype agreed with that expected assuming Mendelian inheritance. However, significant deficiencies of heterozygotes were observed in six out of eight 2-2 x 1-2 crosses made in 1974 and 1976. As demonstrated in the results by tests made for other loci, contamination between crosses may be eliminated as a cause of the anomalies.

Purdom et al. (1976) had reported a significant deficit of 1-2 heterozygotes in the overall total of seven similar 2-2 x 1-2 crosses carried out in 1972, although when considered individually only one showed this significant deficit. Four of the crosses had suffered severe mortalities during rearing and it was suggested that the 1-2 heterozygote was less fit than the 2-2 homozygote under adverse hatchery conditions. The experiments carried out in this study were an attempt to explore this possible selection effect in greater detail. Due to the scarcity of suitable parents 1976 was the only year in which it was possible to carry out enough 2-2 x 1-2 matings to enable the survival rates of 1-2 heterozygotes with age and at different rearing temperatures to be examined. The results showed that there was no difference in the ratio of 2-2 homozygotes to 1-2 heterozygotes between the age of 10 and 82 days. Survival rates in these 2-2 x 1-2 crosses did not differ from those observed in other matings made at this or other loci, suggesting that the 1-2 heterozygote is neither better nor worse fitted to survive in hatchery conditions. The segregation ratios were the same when larvae were reared at different temperatures. Rearing experiments have shown temperature to affect

the survival of LDH genotypes in the crested blenny, Anoplarchus (Johnson, 1971) but these experiments were carried out in the first few days after hatching and in this species the frequencies of different LDH alleles in natural populations are related to seawater temperature.

Four of the 1972 2-2 x 1-2 crosses discussed by Purdom et al. (1976) were listed by Dando (1974) but no comments on segregation ratios were made.

A lack of suitable females prevented enough 1-2 x 2-2 matings being made to test the survival of 1-2 heterozygotes in the reverse cross. Only one such cross was made, in 1974, when the numbers of offspring, although small, complied with the expected Mendelian ratio. Ward and Beardmore (1977) included data from two 1-2 x 2-2 crosses where again the observed results agreed with the expected but, as above, the numbers of offspring were low.

The possibility of linked deleterious genes remains, as discussed previously for the anomalous PGM results; the same limitations of either proving or disproving their presence apply.

The experiments do suggest that any selection against 1-2 heterozygotes must take place within the first ten days of the life history.

Examples of selection against heterozygotes with increasing age have been recorded for transferrin alleles in the skipjack tuna, Katsuwomus pelamis (Fujino and Kang, 1968) and for esterase polymorphism in the viviparous blenny, Z. viviparus (Christiansen, Frydenberg and Simonsen, 1973; Christiansen, Frydenberg, Gyldenholm and Simonsen, 1974) but these took place in natural populations and over a longer period in the life cycle. Beardmore

and Ward (1977) included this GPI-A locus, along with four others, in their study of heterozygosity levels with age in juvenile plaice populations and reported a decrease in the overall heterozygosity of all five loci between the age of 98 and 190 days; after this time the trend was reversed. However, in all these instances differential migration may have affected the observed genotype frequencies.

(ii) GPI-B: The segregation of offspring in 15 of the 20 crosses involving heterozygotes at this locus was in agreement with assumed Mendelian inheritance. The remaining five showed segregation anomalies which were not reflected, however, at other loci in the same crosses. In four of these crosses no other genetically identical GPI-B cross was made so no corroboration of the results could be made. In the remaining anomaly, a 6-6 x 4-6 cross, two other matings between the same parents gave expected results; as did two similar crosses involving different adult fish. This suggests that in this latter instance the unexpected result may have occurred by chance, a possibility when large numbers of crosses are being tested.

One common factor between three of the irregular crosses was the excess of phenotype 6 homozygotes in the offspring. In fact all five anomalies were caused by either an excess of such homozygotes, an excess of genotypes containing allele 6 or a deficiency of non-allele 6 containing genotypes in the offspring. This could suggest that under the conditions of the experiments positive selection occurs for allele 6. In the adult fish this allele was by far the most common suggesting that if selection at this locus does occur in a natural environment, individuals with this allele are well fitted for survival. However, the segregation

of offspring in ten other crosses, all of which included phenotype 6 homozygotes, was in accordance with Mendelian inheritance and did not indicate any differential fitness.

The survival of heterozygotes between alleles of rare occurrence in the parent fish, i.e. genotype 4-5^a offspring in the 4-6 x 5^a-6 cross and genotype 4-7 offspring in the 4-7 x 4-6 cross does tend to be lower than that of other genotypes in these crosses. However, as these crosses were not repeated it is impossible to substantiate whether or not there is a viability effect.

As with the previously discussed anomalies at other loci the possibility of linked deleterious genes exists but cannot be investigated without further experiments.

It is possible for contamination from GPI-B 6-6 x 6-6 crosses combined with a suitable PGM cross to be the cause of the abnormal results in four of the five instances. However the precautions taken to prevent this and the improbability of such selective mixing occurring by accident make this unlikely.

The 1974 and 1975 data discussed here were included, together with some earlier work first presented by Dando (1974), by Purdom et al. (1976). None of the anomalous offspring ratios were presented, and their data supported Mendelian inheritance at this locus. Ward and Beardmore (1977) included the results of three crosses at this locus; although again the numbers of offspring were small, no deviation from expected ratios was observed.

The single anomalous 6-7 heterozygote found in a 6-6 x 6-6 homozygote cross will be discussed later, when individual anomalies found in other crosses at other loci are considered.

The reason why the offspring from so many homozygous crosses were exmained at these two GPI loci was because this enzyme was stained for on slices of gels run primarily to test for PGM.

(e) 6 PGD

Polymorphism at this locus was very rarely observed in the parent fish. Only crosses between presumed homozygous phenotype 2 individuals were made. All the offspring from 13 such crosses were of phenotype 2. Thus although Mendelian inheritance has not been proved at this locus the allelic control of polymorphism described on page 45 (Adult fish - Results) is supported.

All the tests at this locus were made on crosses primarily carried out to study inheritance of polymorphism at either the G3PDH or NAD.MDH-A loci.

(f) IDH

Experiments at this locus were restricted by the difficulties experienced in typing the adult fish and the low level of polymorphism observed. Only one cross between assumed phenotype 2 homozygotes was tested; all the offspring were of the parental type. The same conclusions made for 6 PGD apply to this enzyme.

Extensive studies have been carried out for IDH inheritance in salmonids where varying accounts of the number of loci and the mode of inheritance have been presented, as described previously on page 67.

(g) ADA

Only five crosses were tested at this enzyme, for reasons previously mentioned (page 45). In all five agreement with Mendelian inheritance was observed.

Therefore, although anomalies were observed throughout the experiments the simplest interpretation of the results for the eight loci studied is that polymorphism for all these enzymes is under the direct genetic control of alleles segregating according to Mendelian principles and behaving as codominants.

LINKAGE

RESULTS

Whenever possible when carrying out the conventional crosses parents suitably polymorphic at more than one locus were chosen to allow tests for linkage to be made in the segregation classes of the offspring. From such crosses linkage data were obtained for the following pairs of loci:

G3PDH . MDH-A

MDH-A . PGM

MDH-A . GPI-B

MDH-A . ADA

PGM . GPI-A

PGM . GPI-B

GPI-A . GPI-B

The offspring from three genetically different types of crosses were considered for linkage. These were:

i. crosses between a double homozygote and a double heterozygote; where all the offspring fall into linkage patterns, e.g.

Parents G3PDH 2-2 x 2-3

MDH-A 2-2 x 2-3

Gametes produced: 2.2 x 2.2 + 3.3 or 2.3 + 3.2.

Linkage categories in offspring: 2-2.2-2 + 2-3.2-3 and 2-2.2-3 + 2-3.2-2.

ii. Crosses between a single heterozygote and a double heterozygote where the former is genotypically distinct from the latter: again all offspring fall into linkage patterns, e.g.

Parents PGM 3-4 x 2-3

GPI-B 4-6 x 6-6

Gametes produced: 3.4 + 4.6 or 3.6 + 4.4 x 2.6 + 3.6.

Linkage categories in offspring: 2-3.4-6 + 3-3.4-6 + 2-4.6-6 + 3-4.6-6 and 2-3.6-6 + 3-3.6-6 + 2-4.4-6 + 3-4.4-6.

iii. Crosses between a single heterozygote and a double heterozygote where both parents have a common heterozygous genotype, e.g.

Parents PGM 3-4 x 3-4

GPI-A 1-2 x 2-2

Gametes produced: 3.1 + 4.2 or 3.2 + 4.1 x 3.2 + 4.2.

Linkage categories in offspring: 3-3.1-2 + 4-4.2-2 and 3-3.2-2 + 4-4.1-2.

Offspring of 3-4.1-2 and 3-4.2-2 genotype can belong to either segregation class and have to beignored.

Table 32 gives details of all the genetical combinations studied for linkage. There were no significant deviations from the null hypothesis of a 1:1 ratio in any of the pairs of segregation classes. There was no evidence therefore of linkage between the pairs of loci which were studied. It was not possible to study linkage between the following loci due to thelack of genetically suitable potential parents:

G3PDH . PGM

G3PDH . GPI-A

G3PDH . GPI-B

G3PDH . ADA

MDH-A . GPI-A

PGM . ADA

GPI-A . ADA

GPI-B . ADA

Table 32 Distribution of offspring between complementary segregation classes to demonstrate any linkage between pairs of loci

Loci	Parental genotypes	No. of matings	Nos. of offspring in the complementary segregation classes	
G3PDH . MDH-A	G3PDH 2-2 x 2-3 MDH 2-2 x 2-3	3	48	54
	G3PDH 2-2 x 1-2 MDH 2-2 x 2-3	1	50	43
MDH-A . PGM	MDH-A 2-3 x 2-2 PGM 3-4 x 4-4	3	83	80
	MDH-A 2-3 x 2-2 PGM 3-4 x 4-4	1	13	9
MDH-A . GPI-B	MDH-A 2-3 x 2-2 GPI-B 6-7 x 2-4	1	60	68
MDH-A . ADA	MDH-A 2-2 x 2-3 ADA 4-4 x 2-4	1	17	20
PGM . GPI-A	PGM 4-4 x 3-4 GPI-A 2-2 x 1-2	3	274	284
	PGM 4-4 x 3-4 GPI-A 2-2 x 2-3	1	101	93
	PGM 3-4 x 3-4 GPI-A 1-2 x 2-2	1	13	7
PGM . GPI-B	PGM 4-4 x 3-4 GPI-B 6-6 x 6-7	1	48	38
	PGM 3-4 x 4-4 GPI-B 6-7 x 6-6	2	113	114
	PGM 3-4 x 4-4 GPI-B 4-6 x 5^a-6	1	76	83
	PGM 3-4 x 3-3 GPI-B 6-7 x 4-6	3	235	251
	PGM 3-4 x 2-3 GPI-B 4-6 x 6-6	1	23	15
	PGM 3-4 x 3-4 GPI-B 4-6 x 6-6	1	29	36
	PGM 3-4 x 3-4 GPI-B 6-6 x 4-6	1	34	38
	PGM 3-4 x 3-4 GPI-B 6-6 x 2-6	2	43	43
GPI-A . GPI-B	GPI-A 2-2 x 1-2 GPI-B 6-6 x 6-7	1	46	40
	GPI-A 2-2 x 1-2 GPI-B 4-6 x 6-7	1	7	5

DISCUSSION

Several of the crosses made were between fish heterozygous at more than one locus and, where technically possible, offspring from these crosses were studied at each of the loci at which the fish was heterozygous.

As explained tests were only made between seven pairs of loci and no evidence of any linkage was found in any of the matings. Purdom et al. (1976) reported no linkage between three of these pairs of loci, PGM.GPI-A; PGM.GPI-B and GPI-A.GPI-B, but details of crosses and segregation classes were not presented. Some of these results combined with more recent material form part of this study. More linkage data, on material supplied from Lowestoft, were included by Ward and Beardmore (1977). No evidence of close linkage was reported for three pairs of loci, PGM.GPI-B again, MDH-A.PGM and MDH-A.GPI-B. The more substantial amounts of data for these five combinations obtained in this study corroborate these earlier reports. The two combinations of loci reported here for the first time, G3PDH.MDH-A and MDH-A.ADA, did not display linkage either.

Whilst the possibility of linkage between five randomly spread loci is unlikely in a species with 24 pairs of chromosomes linkage might have been expected between the two GPI loci, A and B, assuming gene duplication had been achieved by some method of tandem duplication.

Unfortunately suitable crosses could not be made to test for linkage between the G3PDH, 6PGD and ADA loci, as linkage has been reported between G3PDH and 6PGD in Lepomis spp. (Wheat et. al., 1973) and between 6PGD and ADA in Xiphophorus spp. (Morizot et. al., 1977).

GYNOGENESIS

RESULTS

Experiments to produce diploid gynogenetic offspring were performed with females heterozygous at one or more enzyme loci. The objective was to determine which of the three possible methods of diploidisation described in the Introduction, page 6 occurs in plaice and to assess any observed recombination between the centromere and each locus as a measure of the cross-over frequency or map length between the centromere and the gene locus. Offspring were successfully raised to study the enzymes G3PDH, MDH-A, PGM, GPI-A and GPI-B and the results are presented in Tables 33 - 36.

If diploidisation of the egg is restored by the most probable method described in the Introduction, i.e. the retention of the polar body from the second division of meiosis and its fusion with the egg pronucleus, the expected segregation in diploid gynogenetic offspring comprises each type of homozygote and heterozygotes of the parental type. The two types of homozygotes should be equally frequent and this frequency should reflect the extent of recombination, as discussed in the Introduction. It is possible, however, for the frequency of one homozygote to differ from the other either by chance or because of differences in viability *per se* or because of close linkage with a deleterious allele at another locus. The possibility of differential viability of the two homozygotes is unlikely in view of the results for the conventional crosses, where no overall advantages were observed for particular homozygotes. However the occurrence of deleterious or recessive lethal genes is possible, as previously suggested in other anomalous crosses.

Table 33 Distribution of genotypes at the G3PDH locus in gynogenetic diploid offspring

Parent genotype	Year	Fish no.	Offspring genotypes				% recombination	
			1-1	2-2	1-2	2-3	on both homozygotes	on the most frequent homozygote
1-2	1978	X	6	2	3		27.3	20.0
		CXII	16	15	20		39.2	38.5
Totals			22	17	23		37.1	34.3
2-3	1978	CI				2		
Overall totals			22	17	23	2	39.1	

Table 34 Distribution of genotypes at the MDH-A locus in gynogenetic diploid offspring

Parent genotype	Year	Fish no.	Offspring genotypes			% recombination	
			2-2	3-3	2-3	on both homozygotes	on the most frequent homozygote
2-3	1975	IX	17	24	23	35.9	32.4
		XI	2	5	9	56.25	47.4
		XII	51	95	92	38.7	32.6
	1976	II	26	18	13	22.8	20.0
		CXIX	2	6	2	20.0	14.3
	1977	LXVII	4				
Total			102	148	139	35.7	32.0

Table 35 Distribution of genotypes at the PGM locus in gynogenetic diploid offspring

Parent genotype	Year	Fish no.	Offspring genotypes					% recombination	
			1-1	3-3	4-4	1-3	3-4	on both homozygotes	on the most frequent homozygote
1-3	1976	I*	5	24		93		76.2	66.0
3-4	1975	I		6	10		43	72.9	68.3
		II		13	19		139	78.9	78.2
		III		-	1		7	87.5	-
		IV		5	12		45	72.6	65.2
		XI		-	-		8		
	1976	II*		15	18		201	85.9	84.8
		CXX		-	-		2		
		XXVII		-	-		4		
		LII		-	1		6		
	1978	CIV		1	2		5	62.5	55.6
Totals				40	63		460	81.7	78.5
Overall totals			5	64	63	93	460	80.7	81.2

*Not the same fish as in 1975; the same numbers were used every year.

Table 36 Distribution of genotypes at the GPI-B locus in gynogenetic diploid offspring

Parent genotype	Year	Fish no.	Offspring genotypes					% recombination	
			4-4	6-6	7-7	4-6	6-7	on both homozygotes	on the most frequent homozygote
4-6	1975	III	3	4		1		12.5	11.1
		XV	68	129		59		23.0	18.6
Totals			71	133		60		22.7	18.4
6-7	1976	II		110	100		24	10.3	9.8
Overall totals			71	243	100	60	24	16.9	14.7

Where differences occur between homozygote frequencies, it may be more realistic to assess recombination levels on the basis of the more frequent homozygote.

In many crosses the numbers of offspring were very low. This was due to the technical difficulty of gynogenesis and, in particular, the poor quality of eggs removed from trawl-caught females. Poor quality eggs seem unable to withstand the rigours of cold shocking. Only at the PGM and GPI-B loci were large numbers of larvae tested due to a combination of satisfactory electrophoretic identification of very young larvae and the occurrence of genetically suitable females.

Each locus will be considered individually in more detail.

(a) G3PDH

Offspring were obtained from three females (Table 33). Only one fish, 1978 number CXII, produced a large number of offspring. The other two produced 11 and 2 offspring respectively. The frequencies of heterozygotes in the offspring from female CXII were 39.2% and 38.5% respectively when either both classes of homozygote or only the more common were considered. The total offspring from the females of phenotype 1-2 gave frequencies of 37.1% and 34.3% respectively.

There was no significant deviation from the expected distribution of the two homozygous classes either in the offspring from females X and CXII or in the total offspring from both of these females.

Inclusion of the two heterozygous larvae from the sole female of phenotype 2-3 increased the frequency of heterozygotes to 39.1% of the overall number of offspring tested at this locus.

A comparison of the distribution of homozygotes and heterozygotes between the two larger broods showed no heterogeneity (X^2 = 9.55, D.F. = 1, 0.50 > P > 0.30).

(b) MDH

Progeny from six females of phenotype 2-3 were tested at the MDH-A locus (Table 34). The overall frequency of heterozygotes in the offspring was 35.7% on the basis of both homozygous classes and 32.0% on the most common homozygote. However, heterozygote frequency amongst the offspring of individual females ranged from 20.0% to 56.25% on the basis of both classes of homozygote and from 14.3% to 47.4% on the most common. The extreme values, however, came from small groups of offspring.

Comparisons were made in each brood between the numbers of homozygotes in each class. For four of these the distribution did not deviate significantly from the expected 1:1 ratio; the exceptions were in the offspring from 1975 female number XII, where there was a deficiency of phenotype 2 offspring (X^2 = 13.26, D.F. = 1, 0.001 > P), and in the offspring from 1977 female number LXVII, where there was a deficiency of phenotype 3 offspring (X^2 = 4.00, D.F. = 1, 0.05 > P > 0.02), but only four offspring were tested in this brood. The deficiency of phenotype 2 offspring was also reflected in a significant deviation from expected equality in the distribution of homozygotes overall (X^2 = 8.46, D.F. = 1, 0.01 > P > 0.001). Removal from the data of the offspring from female XII eliminated this deviation.

Despite these differences between homozygous classes a comparison of the distribution of homozygotes and heterozygotes between the five larger groups of data showed no heterogeneity (X^2 = 8.97, D.F. = 4, 0.10 > P > 0.05).

A total of 67 offspring from six broods from phenotype 2 females were also tested in 1978. All were of the expected phenotype 2.

(c) PGM

Offspring were reared from one female of phenotype 1-3 and ten females of phenotype 3-4 (Table 35). Several of the females produced only a few individuals each.

When both classes of homozygote were included the frequency of heterozygotes in the progeny of the female of phenotype 1-3 was 76.2%. This value did not differ significantly from the corresponding frequency in the progeny from the females of 3-4 phenotype which was 81.7% (X^2 = 1.93, D.F. = 1, $0.20 > P > 0.10$). This combined value for both groups of offspring was 80.7%. When the heterozygote frequency is estimated only on the basis of the most frequent homozygote within each type of brood the distribution of offspring from the female of phenotype 1-3 is significiantly different from that from offspring of the females of phenotype 3-4 (X^2 = 4.91.D.F. = 1, $0.05 > P > 0.02$). Similarly, if the estimates are based on the common homozygote 3-3 there is again a significant difference between distributions (X^2 = 13.54, D.F. = 1, $0.001 > P$). However, irrespective of these differences, the overall frequency of heterozygotes on the most common phenotype 3 homozygote class was 81.2% and almost identical to the overall value on all homozygotes.

There were significant deviations from the expected 1:1 ratio of the two classes of homozygote in both groups of progeny. An excess of phenotype 3 offspring was present in the progeny of the female of phenotype 1-3 (X^2 = 12.45, D.F. = 1, $0.001 > P$) and an

excess of phenotype 4 offspring was found in the progeny from the females of phenotype 3-4 (X^2 = 5.14, D.F. = 1, 0.05 > P > 0.02). In the latter case only the summed data showed this deviation; the progeny from each individual female did not differ from the expected ratio.

A contingency test of homozygote and heterozygote distribution between offspring from each female showed homogeneity (X^2 = 14.51, D.F. = 8, 0.10 > P > 0.05). The numbers of offspring from two females in 1976, numbers CXX and XXVII were too small to be included.

A total of 100 offspring from four females of phenotype 4 were also tested, two broods in 1975 and two in 1978. All the offspring were of the expected phenotype 4.

(d) GPI

(i) GPI-A

A total of 60 offspring from one female of phenotype 1-2 were tested in 1978. The distribution of offspring was phenotype 1, 20; phenotype 2, 13; phenotype 1-2, 27. There was no significant deviation from the expected 1:1 ratio of the homozygous classes.

The frequency of phenotype 1-2 heterozygotes in this total was 45% and when only the more common phenotype 1 homozygote class was considered it was 40.3%.

A total of 620 offspring from 16 broods from phenotype 2 females were also tested, five in 1975, ten in 1976 and one in 1978. All were of the expected phenotype 2.

(ii) GPI-B

Offspring were tested from two females of phenotype 4-6 and one female of phenotype 6-7 (Table 36). The frequencies of

heterozygotes found in the totals for the two types of brood were 22.7% from 4-6 females and 10.3% from the 6-7 female. These heterozygote distributions were significantly different (X^2 = 14.36, D.F. = 1, 0.001>P). Similarly, when only the most common class of homozygote for each type of brood was considered a significant difference in distribution was again found (heterozygote frequencies 18.4% and 9.8% respectively, (X^2 = 4.08, D.F. = 1, 0.05>P>0.02).

There was a significant deviation from the expected 1:1 ratio in the numbers of homozygotes in the offspring of the females of phenotype 4-6 (X^2 = 18.84, D.F. = 1, 0.001>P) caused by the contribution of female number XV (X^2 = 18.89, D.F. = 1, 0.001>P), but the distribution was as expected in the offspring of the female of phenotype 6-7.

Pooling all the data gives heterozygote frequencies of 16.9% and 14.7%, respectively, when either all the homozygotes or only the most common are considered.

A total of 182 offspring from ten broods from phenotype 6 females were tested, one in 1975, five in 1976 and four in 1978. All were of the expected phenotype 6 except for one phenotype 4-6 offspring in one of the 1978 broods. Once again the simplest interpretation of this single anomaly is that of a mutation to allele 4 from allele 6.

(e) 6PGD

In 1977 a total of 145 offspring from two broods from phenotype 2 females were tested. All were of the expected phenotype 2. No suitable heterozygous females were ever available at this locus.

DISCUSSION

The results of the study of the inheritance of five enzyme systems using induced gynogenesis complied with two concepts: first that codominant alleles control the polymorphism at each locus and, secondly, that the diploid state of the offspring is caused by the retention of the second polar body. The former has been demonstrated already from the results obtained from conventional crosses: the latter, already suggested by Purdom and Lincoln (1973), was confirmed in these experiments by the occurrence of each type of homozygote and the respective heterozygote at various frequencies in broods from heterozygous females. If diploidy arose by involvement of the first polar body a minimum ratio of 1:2:1 for homozygote:heterozygote: homozygote would be expected. Each homozygote would represent a recombined chromosome pair, therefore even 100% crossing-over between the centromere and a locus should only produce a maximum of 50% homozygotes in the offspring. If diploidy arose through fusion of the products of first mitosis, the offspring would be all homozygous.

Anomalies did occur in the segregation ratios: of genotypes in some broods. These fell into three categories:

(i) significant deviations from the expected 1:1 ratio in the homozygote classes;

(ii) significant differences in the distribution of heterozygotes in offspring from different females;

(iii) higher than expected frequencies of heterozygotes in the offspring.

The possible explanations of the first of these anomalies have already been mentioned, namely chance differences in viability

or linkage with a deleterious allele at another locus. Similar phenomena may account for the second type of discrepancy between broods. In order to identify the third type of anomaly the chromosomal behaviour required to produce a heterozygous gynogenetic diploid needs further explanation. As the results have shown that diploidy is caused by retention of the second polar body each heterozygous gynogenetic offspring represents a recombined chromosome pair; the result of a cross-over event or events between the centromere and the locus. A single cross-over will produce heterozygosity distal to the cross-over in the pair of chromosomes in the egg. Where two cross-overs occur between the locus and the centromere, but only involving one pair of chromatids no recombination will be apparent although some heterozygosity is nevertheless produced. However, where more complicated crossing over occurs involving all four chromatids the situation becomes more complex. A heterozygote will be produced whenever a cross-over is added to a situation which otherwise would produce a homozygote. In the alternative situation when a cross-over is added to what would otherwise be a heterozygote the result is more complicated. Of the four possibilities two lead to homozygosity and two to retain heterozygosity. The probability of heterozygosity for a particular number of cross-overs may be determined by the following equation (Purdom et al., 1976):

$$P_x = 1 - \tfrac{1}{2} P_{(x-1)}$$

where x = no. of cross-overs

e.g. let x = 3

$$\therefore P_3 = 1 - \tfrac{1}{2} P_2.$$

The proportion of heterozygotes expected after two cross-overs between the centromere and the locus is 50%

$$\therefore P_3 = 1 - \tfrac{1}{2}\cdot\tfrac{1}{2}$$

$$= \tfrac{3}{4} \text{ or } 75\% \text{ of the offspring.}$$

As the number of cross-overs increases this figure tends towards a maximum of 67%.

The occurrence of any of these three anomalies will be commented on as the results for each locus are discussed in detail.

The recombination frequencies observed by induced gynogenesis may be used to obtain estimates of map length between the centromere and the locus. As already stated each heterozygote represents one recombined chromosome pair produced by an uneven number of cross-overs between the centromere and the locus. By comparison, in conventional recombination between pairs of linked loci, a crossover event produces only a single recombined chromosome in the offspring. Therefore the recombination frequencies observed in gynogenesis experiments represent twice the values obtained in conventional recombination analyses as used for chromosome mapping. Estimates of the map length between the centromere and each locus may be obtained by halving the observed recombination frequencies.

The results for each locus will be discussed independently.

(a) G3PDH

The results for this locus were limited by the need to rear larvae to metamorphosis in order to obtain satisfactory electrophoretic results. In the two broods large enough to allow statistical analysis, the frequency of recombination was consistent. There was no significant deviation from the expected 1:1 ratio of the homozygote classes either in each brood or the pooled data.

The numbers of heterozygotes observed when recombination was considered on either both or only the most common of the homozygote classes did not significantly differ (X^2 = 0.30, D.F. = 1, 0.70>P>0.50). Taking the mean of the heterozygote proportions and dividing by two, a map length of 18 map units may be estimated for G3PDH, assuming only one crossover between centromere and locus, see Table 37.

(b) MDH-A

A wide range of recombination frequencies was obtained from the different broods examined at this locus. Despite this there were no significant differences in the proportions of heterozygotes observed in the broods large enough for statistical analysis. A significant deficiency in one of the homozygote classes in one large brood was reflected in the overall homozygote distribution. No examples of deficiencies of this particular phenotype were observed in any of the appropriate conventional crosses. The possible reasons for such an anomaly have already been discussed. There was no significant difference between the distribution of recombinants when either both or the most common of the homozygote classes were considered (X^2 = 0.21, D.F. = 1, 0.70>P>0.50). Taking the mean of these recombination frequencies and again assuming only one cross-over an estimate of the map length between this locus and the centromere is 17 map units (see Table 37).

(c) PGM

More experiments were carried out for this enzyme than for any of the others, for reasons explained previously, see page 96 Significant deviations from the expected 1:1 ratio were observed in the homozygote classes of broods from both phenotype 1-3 and phenotype 3-4 females. For the latter this was only apparent in

Table 37 Summary of observed recombination frequencies in gynogenetic offspring and an estimate of map length between each locus and the centromere

Locus	On both homozygotes			On one homozygote			Mean Map Length
	n	Observed recombination (per cent)	95% conf. level (± 2 x s.e.)	n	Observed recombination (per cent)	95% conf. level (± 2 x s.e.)	
G3PDH	64	39.1	± 12.2	33.5	34.3	± 16.4	18.4
MDH-A	389	35.7	± 4.9	217.5	32.0	± 6.3	16.9
PGM	685	80.7	± 3.0	340.5	81.2	± 4.6	40.5
GPI-A	60	45.0	± 12.9	33.5	40.3	± 17.00	21.3
GPI-B	498	16.9	± 3.4	285	14.7	± 4.6	7.9

the pooled data and not in individual broods from different females. When these females were involved in conventional crosses the segregation ratios of the offspring were always as expected. Although superior fitness of phenotype 4 homozygotes over phenotype 3 homozygotes and of phenotype 3 homozygotes over phenotype 1 homozygotes may be suggested by these gynogenetic results this is not supported by the results from the conventional crosses where there was no consistent bias in favour of a particular homozygous phenotype. Previously advanced reasons may account for the anomalies, see page 97

These anomalies in the homozygote ratios caused some differences in heterozygote distributions between the offspring of the phenotype 1-3 and phenotype 3-4 females but when both classes of homozygote were included these distributions did not significantly differ. The overall values of 80.7% heterozygotes on both homozygotes and 81.2% heterozygotes on the most common phenotype 3 homozygote are very close together and may be combined to give a mean estimate of map length between this locus and the centromere. These heterozygote frequencies are significantly higher than the theoretical maximum probability of heterozygosity of 67% as described on page 121.It may be that a loss of homozygotes due to lethal or deleterious genes can account for this but although some anomalies in segregation ratios were observed in the results of the conventional crosses the majority of such crosses do not substantiate such an explanation. Heterozygote frequencies higher than 67% were observed not occasionally but consistently at this locus in the broods of gynogenetic diploids. Discounting involvement of the first polar

body in diploidisation positive interference could account for the apparent high level of recombination. Taking the mean of the two overall recombination percentages and assuming total positive interference after one cross-over a map length of 40 map units between the PGM locus and the centromere is obtained (see Table 37).

(d) GPI

(i) GPI-A

Only one brood was examined at this locus. The scarcity of genetically and physiologically suitable females prevented more experimental work. There was no deviation from the expected 1:1 ratio of homozygotes. The distributions of heterozygotes when either both or only the most common of the homozygote classes were considered were not significantly different (X^2 = 0.26, D.F. = 1, $0.70 > P > 0.50$). Taking the mean of these recombination frequencies the lowest estimated map distance between the GPI-A locus and the centromere is 21 map units, see Table 37.

(ii) GPI-B

At this locus, as at the previous one, the experiments were limited by the availability of suitable material. Only three broods were tested and one of these contained very few offspring. There were significant differences in the distribution of heterozygotes between the two larger broods when either both or only the most common of the homozygote classes was included. In one of the broods there was also a significant deviation from the expected 1:1 ratio of the homozygotes. This was caused by the excess of phenotype 6 homozygotes; an anomaly in keeping with the results observed in the conventional crosses. However with only these limited amounts of data available further studies are

required before any conclusions may be drawn. Despite the differences between broods, in the pooled data there was no significant difference between the heterozygote distributions on either one or both homozygotes (X^2 = 0.01, D.F. = 1, 0.90>p> 0.80). As the differences mentioned above were only between single broods and unsubstantiated by further experiments the mean of these overall heterozygote frequencies was used to estimate the map length between this locus and the centromere. A value of 8 map units was obtained, see Table 37.

The data discussed so far result from experiments where some success was achieved in inducing diploid gynogenesis. Many more experiments were attempted but failed due to mortalities during embryonic growth. The majority of the failures were associated with the cold shocking procedure. The quality of eggs from hand-stripped females was often poor and these eggs, although retaining viability during normal fertilisations, appeared unable to withstand the rigours of sudden temperature changes. Further gradual losses occurred throughout the development of the egg, similar to those experienced in the conventional crosses but other heavy mortalities took place either during or shortly after hatching. Many gynogenetic diploid larvae developed with severe physical deformities placing restrictions upon movement and feeding. As a consequence these larvae could not survive after utilisation of the yolk sac.

Map distances for three of the five loci discussed here were presented by Purdom et al. (1976). The estimates are almost identical; as may be expected as the data used by Purdom et al. (1976) are common to both studies. Detail of the estimates are as follows:

	Distance in map units	
	This study	Purdom et al. (1976)
MDH-A	17	19
PGM	40	41
GPI-B	8	9

Following the work of Barker (1972) who counted 24 telocentric pairs of chromosomes in plaice, with the length of the largest being approximately twice that of the smallest, Purdom et al. (1976) suggested, that, if the PGM locus is terminal on the largest chromosome plaice may have a minimum estimated total map length of 720 units.

The cross-over values obtained in the experiments discussed here may be used to indicate the efficiency of induced gynogenesis in the production of inbred lines of plaice.

In self-fertilisation, the coefficient of inbreeding (F) is 0.5 (Falconer, 1960) and for successive generations of selfing $F = \frac{1}{2}(1 + F_p)$ where F_p is the coefficient of inbreeding of the parent. The experiments carried out have shown induced diploid gynogenesis to be caused by the retention of the second polar body, i.e. the retention of sister chromatids, and this is equivalent to total inbreeding of the parent ($F_p = 1$). This value will be reduced by the degree of crossing over (x) between non-sister chromatids in the first meiotic division. Therefore, substituting in the equation, for gynogenetic diploids

$$F = \frac{1}{2}(1 + (F_p - x))$$
$$= \frac{1}{2}(1 + (1 - x))$$
$$= \frac{1}{2}(2 - x)$$

Taking the mean of the cross-over frequencies obtained for the five loci, see Table 37 , $x = 0.21$ suggesting that for first generation gynogenetic diploid plaice the coefficient of inbreeding

is $\frac{1}{2}$ (2 - 0.21) or 0.895. This value is equivalent to between three and four generations of conventional sibmating. A high coefficient of inbreeding may explain the heavy mortalities experienced in some of the experiments. However, it also emphasises the important role induced gynogenesis may play in the breeding strategy of commercial fish cultivation. A single generation of gynogenesis would eliminate the need for many generations of sibmating and the inherent years of fish husbandry. In plaice the female is the heterogametic sex (Purdom, 1969) therefore gynogenesis produces offspring of both sexes allowing crosses between different inbred lines. The low survival rates in gynogenetic broods may be counteracted by the high fecundity of marine flatfish hence only small numbers of broodstock need to be maintained. Crosses between gynogenetic individuals could be used to examine for heterosis.

The results in this study may be compared to studies made in the USSR on induced gynogenesis in the carp, Cyprinus carpio (Golovinskaia, 1968; Cherfas, 1977; Cherfas and Truveller, 1978). The inheritance at seven loci was examined in broods of gynogenetic diploids; three loci were identified by electrophoretic techniques and the remaining four controlled physical characteristics of scale pattern and pigmentation. Recombination frequencies greater than 67% were reported for three of the seven loci but the results were complicated by lethal combinations, dominance and ontogenetic effects at some of the loci. Initially Golovinskaia (1968) reported 100% and 30% heterozygote frequencies at two loci, N-n and S-s respectively, controlling scale pattern but these results were complicated by a lethal N-N homozygote at the former and dominance of the S allele at the latter. However the numbers of offspring

studied were small and apart from suggesting that diploidy was restored in induced gynogenesis by retention of the second polar body, which is not proved by 100% heterozygosity, no further interpretations were made. Cherfas (1977), in a more extensive account, also reported a high heterozygote frequency, 97.3%, at the N-n locus but a reduced frequency, 4.8%, at the S-s locus. Heterozygote frequencies of 70.0% and 73.9% were obtained for two new loci, D-d and L-l respectively, controlling pigmentation, but again there were complications, a lethal L-L homozygote and dominance of the D allele. Consequently attribution of genotypes into specific segregating classes was again in part arbitrary. Electrophoretic data for a transferrin and two esterase loci all gave lower heterozygote frequencies (Cherfas and Truveller, 1978). At the transferrin locus an overall heterozygote frequency of 13.1% was observed but for individual broods this value varied from 0.0-43.8%; similarly a discrepancy occurred when the results were divided into '0' and '1' group fish, heterozygote frequencies 16.6% and 1.1% respectively. No explanation for these variations was offered and by disregarding those broods containing few individuals and those with high recombination frequencies a distance of three map units between the centromere and the transferrin locus was suggested. Considering all the data a greater distance of 7 map units is obtained. Heterozygote frequencies of 26.8% and 9.1% were reported for two independently controlled esterase loci; no differences between age groups were observed. Map distances of 13 and 5 map units may be estimated between these loci and the centromere.

In a separate study Nagy, Rajki Horvath and Cranyi (1978) reported finding 6.3% heterozygotes at the transferrin locus in

in diploid gynogenetic carp; a value similar to that suggested by Cherfas and Truveller (1978). Although 143 offspring were tested no details of genotypes or broods were given and no other protein or enzyme loci were studied.

Thus of the seven loci studied in gynogenetic carp three gave recombination frequencies greater than the postulated maximum of 67%. Cherfas (1977) suggested that, as proposed here for the high recombination frequency, at the PGM locus in plaice, interference may cause these high results. Although, as explained here, the data are complicated, this interpretation may be correct. By considering all these data a mean cross-over frequency of 0.21 can be estimated for carp; a figure identical to that obtained for plaice in this study.

TRIPLOIDY

RESULTS

Experiments to produce triploid larvae were made; where possible parents of the genotypes necessary to provide details of the method of triploidisation were selected. The three possible methods of inducing triploidy are the same as those postulated for induced gynogenetic diploids in the Introduction, page 6, with, in each case, the inclusion of the chromosomal contribution of the male gamete. As discussed for induced gynogenesis the segregation of offspring into recombinant and non-recombinant classes can determine the method occurring in these plaice experiments.

Crossover frequency estimates obtained may also supplement the data from the induced gynogenesis experiments.

Many triploid crosses were attempted but unfortunately the majority failed, possible due to poor egg quality. Nevertheless a reasonable number of offspring were reared and successfully typed for segregation at four loci, MDH-A, PGM, GPI-A and GPI-B. The results are listed in Tables 38, 39 and 40; the data from all crosses between like homozygotes are pooled, as are the results of multiple crosses involving the same parent fish.

None of the crosses at the GPI-A locus were between parents of suitable genotype to produce identifiable recombination classes in the offspring, see Table 38. These crosses were made because of genetical interest at another locus and staining for GPI-A was incidental.

For two of the remaining three loci, GPI-B and MDH-A the identification of recombinants was possible only by the occurrence of differential staining between individuals. For instance, at the

Table 38 Distribution of genotypes at the GPI-A and GPI-B loci in induced triploid offspring

Parent genotype	Year	Cross no	Offspring genotypes					
			2-2-2	1-2-2	2-2-3	6-6-6	4-4-6	4-6-6
1. GPI-A								
2-2 x 2-2	1975	3 crosses	8					
	1976	30T	56					
total			64					
2-2 x 1-2	1976	39T+42T	61	60				
2-2 x 2-3	1976	22T	53		53			
2. GPI-B								
6-6 x 6-6	1975	2 crosses				4		
	1976	4 crosses				291		
total						295		
4-6 x 6-6	1975	21T				2	1	1

Table 39 Distribution of genotypes at the MDH-A locus in induced triploid offspring

Parent genotype	Year	Cross no	Offspring genotypes			% recombination	
			2-2-2	2-2-3	2-3-3	On both non-recombinant classes	On the most frequent non-recombinant class
2-2 x 2-2	1977	4T	3				
2-3 x 2-2	1975	16T+26T	5	11	8	33.3	26.7
		19T	1	4	4		
totals			6	15	12	36.4	44.4

Table 40 Distribution of genotypes at the PGM locus in induced triploid offspring.

Parent genotype	Year	Cross no.	Offspring genotypes							% recombination	
			4-4-4	1-1-4	1-3-3	1-3-4	1-4-4	3-3-4	3-4-4	On both non-recombinant classes.	On the most frequent non-recombinant class
4-4 x 1-4	1975	21T	3				1				
1-3 x 4-4	1976	22T		34		24		55		21.2	17.9
		30T		4		46		6		82.1	79.3
		39T+42T		5		108		9		88.5	85.7
totals				43		178		70		61.2	56.0
3-4 x 1-4	1975	32T	6		4	8	2		7	55.6	48.4
3-4 x 4-4	1975	*DT5+24T	8						40		
		DT7	1						1		
totals			9						41		

* 3-3-4 and 3-4-4 offspring not distinguishable in these two crosses. Also one genotype 2-4-4 offspring found in cross number DT5.

GPI-B locus, the 4-4-6 and the 4-6-6 offspring were distinguished by the fact that, in the former, the isozyme 4 band stained more intensely than the isozyme 6 band, whereas the reverse was the case in the latter offspring.

The only cross producing a recombination class at the GPI-B locus contained only four offspring but it did demonstrate a crossover event (phenotype 4-6-6) and confirm triploidy (parents GPI-B 4-6 x 6-6; offspring 6-6-6 2, 4-4-6 1 and 4-6-6 1). More successful crosses were made for the MDH-A locus (Table 39); offspring from three crosses between 2-3 and 2-2 genotypes contained 36.4% recombinants on both non-recombinant classes and 44.4% on the most common non-recombinant class. This suggests a crossover frequency of about 20% between the locus and the centromere which is similar to the value of 17% obtained from the diploid gynogenetic experiments, page 122.

The results of all the triploid crosses made at the PGM locus are listed in Table 40. Four different genotypic pairings of parents were used but the offspring from only two of these produced identifiable recombination classes. One set of data, from genotype 3-4 x 1-4 parents gave recombinant percentages of 55.6 on both classes of non-recombinant homozygote and 48.4 on the most common, suggesting a crossover frequency of about 26%. This is lower than the value of 40% obtained from diploid gynogenesis but the numbers of triploid offspring were not large. The results of four crosses made between genotype 1-3 x 4-4 parents contained more offspring, but suggest a similar value for crossover frequency, i.e. about 29%; again lower than from diploid gynogenesis. However, a considerable discrepancy was found in the distribution of non-

recombinants and recombinants between the four crosses, (X^2 = 124.68, D.F. = 2, 0.001>P). This was due to a large excess of non-recombinants in 1976 cross 22T. As the same female was used for all four of the crosses it may be suggested that the male used in this particular mating contributed a factor which in conjunction with PGM 1-3 alleles proved deleterious. However, eggs stripped from the female at the same time as those used in cross 22T were also used in a gynogenetic diploid cross, 1976 number 22H, which also produced a highly irregular segregation within offspring classes. Excluding the results of cross 22T the offspring from the remaining three 1-3 x 4-4 crosses gave recombination values of 86.5% on both non-recombinant classes and 83.7% on the most common; suggesting a crossover value of about 43% which corresponds to that of 40% obtained by diploid gynogenesis.

Combining the data from the 3-4 x 1-4 cross and the 1-3 x 4-4 crosses gives overall recombination values of 60.7% on both classes of non-recombinant and 58.0% on the most common. Removal of the offspring from cross 22T modifies these values to 82.4% and 84.9% respectively; giving a crossover frequency of 42% which is very similar to the value obtained from diploid gynogenesis.

Another anomaly in the PGM results was the occurrence in 1975 cross DT5 of a single offspring with genotype 2-4-4. As previously mentioned on page 82 allele 2 was not present in the 1975 parental gene pool and the simplest interpretation is that this anomaly represents a mutation to allele 2 from either allele 3 or allele 4.

Table 41 summarises results at the MDH-A and PGM loci. The values of 20 and 42 map units for the distances of the MDH-A and PGM loci respectively from the centromere are similar to the values obtained from diploid gynogenesis.

Table 41 Summary of observed recombination frequencies in triploid offspring and an estimate of map length between each locus and the centromere.

Locus	On both non-recombinants			On the most frequent non-recombinant			Mean Map length
	n	Observed recombination	95% confidence level (±2 x s.e.)	n	Observed recombination	95% confidence level (±2 x s.e.)	
MDH-A	33	36.4	± 16.8	21	44.4	±21.7	20.2
PGM (i) Overall	318	60.7	± 5.5	166.5	58.0	± 7.7	29.7
(ii) Without 22T	205	82.4	± 5.3	99.5	84.9	± 7.2	41.8

DISCUSSION

By comparison with the conventional and gynogenetic data the numbers of triploid offspring examined were small. The low levels of heterozygosity observed in the adult fish at all the loci examined in this study, with the exception of PGM and ADA, restricted the numbers of matings made between phenotypes capable of producing triploid recombinant offspring with three alleles. However induced triploidy was studied at four loci although with respect to two of these, GPI-A and GPI-B, the majority of the crosses were either between like homozygotes or between a homozygous female and a heterozygous male and consequently the offspring produced were unsuitable for determining recombination frequencies. The results at these two loci were obtained mainly from crosses made to study recombination at another locus. By observing differential staining it was possible to identify recombinant classes at the GPI-B and MDH-A loci in crosses between heterozygous females and homozygous males. The numbers at the GPI-B locus were too small for any statistical interpretation and merely demonstrated triploidy but at the MDH-A locus the proportion of recombinants was almost identical to that observed for this locus in gynogenetic diploids; hence confirming the estimate of map length for MDH-A made from these results. The greater amount of data available for the PGM locus includes crosses between a heterozygous female and a male homozygous for a third allele. The occurrence of offspring possessing all three alleles in the recombinant class demonstrates not only the triploid state but also that this is not caused by any fusion of products of the first mitosis; only one allele from the female would be present if this were the case. The occurrence of different frequencies of these

three-alleled individuals at different loci and not always above the minimum of 50% as would have been expected if the first polar body was involved in triploidisation confirms that triploidy was obtained by a combination of the second polar body with the egg and sperm pronuclei. If the one cross which produced an anomalous result is ignored, as explained previously, (results page 136) the recombination value obtained for PGM from segregation in induced triploid offspring is almost identical to that obtained from gynogenetic diploids; thus confirming the estimate of map length already made for this locus.

In this particular study induced triploidy was used as an alternative to gynogenetic diploidy to determine crossover frequencies. However the production of triploids may be important in commercial fish cultivation since triploid fish cannot successfully produce viable gametes by meiosis. Males may develop gonads and attempt the production of spermatozoa but are unsuccessful while triploid females are unable to produce viable oocytes, thus retarding the development of a large gonad. Consequently in these sterile females the metabolic effort required for gonad production may be diverted to give increased growth rate and greater productivity of saleable protein. Purdom (1972) suggests that, as the plaice has indeterminate growth size, regulatory mechanisms other than those concerned with the rates of feeding and conversion may be absent and that, as in plants, the growth rate might be proportional to the degree of ploidy. Consequently, as in plant breeding, polyploidy may be an important role in commercial fish cultivation.

INDIVIDUAL ANOMALOUS F1 PHENOTYPES

The appearance of unexpected individual phenotypes in nine independent instances has already been mentioned (see pages 80, 92, 118) seven involved the PGM locus and two the GPI-B locus. As already stated contamination would be an unlikely explanation of these anomalies and in most instances could be eliminated by the absence of a suitable phenotype in the available parental pool. As previously concluded the simplest interpretation is that these anomalies represent mutations. The possibility of detecting mutations is simplified by the codominant nature of the alleles but the estimation of mutation rates is not straightforward since some mutations will be observable, others not. Thus in a PGM cross 1-2 x 1-1 a mutation to any allele other than 1 or 2 will always be obvious, a mutation to allele 2 in the germ cells of the 1-1 parent will be detectable in half the zygotes which received it and a mutation to allele 1 in the germ cells of the 1-2 parent will not be detectable at all. The total numbers of gametes which could carry potentially identifiable mutations to each allele are listed in Table 42, together with the observed numbers of mutations. The mutation rates, computed by the number of mutations divided by the mean number of tested gametes, were 8.40×10^{-4} mutations per gamete for PGM and 1.95×10^{-4} mutations per gamete for GPI-B.

No mutations were observed at the other six loci studied and estimated values of less than 1.6×10^{-3}, 3.1×10^{-4}, 1.15×10^{-4}, 4.44×10^{-4} and 5.75×10^{-3} mutations for the G3PDH, MDH-A, GPI-A, 6PGD and ADA loci respectively were obtained; the numbers

Table 42 Estimated numbers of gametes in which mutations could have been identified at the PGM and GPI-B loci and the presumed mutations to each allele

Locus	Allele									
	1	2	3	4	5[a]	5	6	7[a]	7	8
PGM										
Estimated numbers of gametes under test	10 795	12 888.5	2 664	2 171	-	13 169	-	-	-	-
Number of presumed mutations	-	4	2	1		-				
GPI-B										
Estimated number of gametes under test	11 935	11 677	11 935	9 061	11 696.5	11 935	-	11 935	10 643.5	11 935
Number of presumed mutations	-	-	-	-	-	-	-	-	1	

Mutation rate/gamete = No. of mutations $\div$ $\frac{\text{Total no. of potentially identifiable mutations}}{\text{No. of alleles}}$

$\therefore$ For PGM mutation rate = 8.40×10^{-4} mutations per gamete

For GPI-B mutation rate = 1.95×10^{-4} mutations per gamete

of gametes under test at the IDH locus were too low for any estimation to be made. These estimates may be compared to those submitted by Purdom et al. (1976) made from less data but including one mutation at the GPI-A locus from data not available for this thesis. Some of the data of Purdom et al. (1976) obtained in 1974 and 1975 are included in this study. A comparison of the two sets of data is made in Table 43.

Although the estimates vary for some of these loci where no mutations were reported in either study, the estimated mutation rate for the PGM locus suggested by Purdom et al. (1976) is similar to that obtained from the more extensive data available from this study. Although these suggested mutation rates may appear high, an even higher value, 1×10^{-2}, has been reported at the 6PGD locus in the Japanese quail, Coturnix coturnix japonica, (Ohno, Stenius, Christian and Schipmann, 1969). These authors suggest that the use of electrophoretic techniques, identifying a great amount of genetic variation, allows mutations to be much more easily recognised and that the mutation rates of 1×10^{-5} obtained in other vertebrates from physical parameters may be an under-estimate.

Ohno et. al. (1969) also suggest that there may be some correlation between the mutation rate at a given locus and the number of alleles observed there. However a comparison of the data in Table 43 and Table 8 (page 30) does not support this idea.

Table 43 A comparison of estimates mutation rates obtained in this study and those of Purdom et al. (1976)

Locus	Mutation rate	
	This study	Purdom et. al.
G3PDH	$< 1.60 \times 10^{-3}$	$< 9.0 \times 10^{-4}$
MDH-A	$< 3.10 \times 10^{-4}$	$< 1.4 \times 10^{-3}$
PGM	8.40×10^{-4}	1.1×10^{-3}
GPI-A	$< 1.15 \times 10^{-4}$	2.6×10^{-4}
GPI-B	1.95×10^{-4}	$< 2.9 \times 10^{-4}$
6PGD	$< 4.44 \times 10^{-4}$	-
ADA	$< 5.75 \times 10^{-3}$	-

POPULATION STUDY

RESULTS

Samples of plaice eggs were collected and returned live to the laboratory from different spawning grounds in 1975, 1977 and 1978. A total of 12 samples were collected representing nine different areas; three areas were sampled twice, either in different years or at different times during the same spawning season, see Table 2A. All these samples, with the exceptions of numbers 11 and 12 from South West Iceland, were collected from spawning grounds on the continental shelf surrounding the United Kingdom.

Upon hatching larvae were reared, whenever possible, until metamorphosis, i.e. to a length of between 10 and 15 mm, and then each individual was electrophoretically tested for up to eight enzyme loci, G3PDH, MDH-A, PGM, GPI-A, GPI-B, 6PGD, IDH and ADA. However in some samples because of the normally high mortality rates present in larval rearing, the numbers of larvae were too low for prolonged husbandry and in these instances tests were made on smaller larvae. When this occurred the size of the individuals limited the number of electrophoretic tests possible, hence not all the fish were tested for all the enzymes; studies were concentrated upon the more easily identified PGM and GPI loci.

Three samples of '0' group metamorphosed fish were collected from different beaches in 1976, see Table 2B. These fish which originated from spawning grounds in the North Sea, were larger, approximately 50 mm in length and were tested for all eight loci.

The results for each locus will be treated independently. A standard procedure was followed throughout the analysis of the data. Firstly the results were examined for any deviation from

Hardy-Weinberg equilibrium within each sample. Then any differences in allele and phenotype frequencies between samples were looked for; in particular between the North and Irish Seas samples and the geographically remote Icelandic samples, and between the '0' group fish and the larvae reared from collected eggs. Finally where homogeneity between samples was found Hardy-Weinberg tests on the overall phenotype distribution were carried out. The statistical analyses followed the principles described for the adult plaice data, see page 27 onwards.

Table 44 gives details of the enzymes studied in each batch of fish.

(a) G3PDH

Not all of the samples were tested for this enzyme for one or other of the following two reasons : (i) As previously mentioned, page 73, the amount of skeletal muscle required to identify this enzyme on the gels could be obtained only from metamorphosed fish. (ii) A reliable technique for this enzyme had not been resolved in 1975.

Details of the distribution of phenotypes in the eight samples tested for G3PDH are listed in Table 45. The expected numbers according to the Hardy-Weinberg Principle for genetic equilibrium are given in parentheses.

X^2 tests were possible for only two of the samples, number 4 from the Gabbard, and number 10 from the Scarborough Ground, both collected in 1977. In the former a significant deviation from Hardy-Weinberg expectation was observed due to an excess of 3-3 homozygotes and a deficiency of 2-3 heterozygotes : in the latter compliance with genetic equilibrium was found. In the remaining

Table 44 Details of numbers of fish tested for each enzyme in plaice reared in the laboratory from eggs collected from different areas and in '0' group plaice collected from three beaches.

Sample number	Date of Collection	Locality	Enzymes						
			MDH-A	PGM	GPI	6PGD	G3PDH	ADA	IDH
1.	30/1/75	Gabbard	252	252	252	252			
2	31/1/75	West Mud Hole	250	250	250	250			
3	9/3/75	Castle Ground (Off Flamborough)	256	256	256	256			
4	26/1/77	Gabbard	279	279	279	279	279	27	27
5	7/2/77	West Mud Hole		135					
6	8/2/77	White Bank	14	83	14	14	14		
7	9/2/77	N.E. Cleaver Bank		82					
8	21-23/2/77	Moray Firth	68	68	68	68		68	
9	4/3/77	Off Maughold Head (Isle of Man)	54	106	54	54	54	54	54
10	12/3/77	Scarborough Ground (Off Flamborough)	165	218	165	165	164	27	113
11	17/4/78	Off Rekjanes I (S.W. Iceland)		286	287				
12	1/5/78	Off Rekjanes II (S.W. Iceland)		40	40	40	40		
13	4/10/76	Lowestoft beach	185	185	185	185	184	185	185
14	14/10/76	Cleethorpes beach	142	142	142	142	137	142	142
15	20/10/76	Filey beach	62	62	62	62	62	61	62

Table 45 G3PDH:- The numbers of types are shown for each of the eight samples. The expected numbers in parentheses assume perfect genetic balance at each position sampled.

Phenotypes	Year and Locality 1977														Overall distribution		Overall distribution - sample 12 from Rekjanes	
	Gabbard (4)		White Bank (6)		Maughold Hd. (9)		Scarboro' Gd. (10)		Off Rekjanes II (12)		Lowestoft (13)		Cleethorpes (14)		Filey (15)					
1 - 1	-	(.75)	-	(.02)	-	(.07)	1	(1.03)	-		-	(.72)	2	(.36)	-	(.06)	3	(2.73)	3	(2.85)
2 - 2	219	(214.24)	12	(12.07)	44	(43.56)	120	(119.52)	39	(39.01)	139	(139.14)	112	(110.43)	51	(49.69)	736	(726.91)	697	(688.39)
3 - 3	7	(1.29)	-	(.02)	-	(.17)	3	(.74)	-	(.01)	-	(.72)	-	(.31)	2	(.33)	12	(3.42)	12	(3.50)
1 - 2	27	(25.43)	1	(.93)	3	(3.59)	24	(22.21)	-		20	(20.00)	9	(12.57)	4	(3.59)	88	(89.15)	88	(88.65)
1 - 3	2	(1.98)	-	(.04)	1	(.22)	-	(1.71)	-		3	(1.44)	1	(.67)	-	(.29)	7	(6.11)	7	(6.32)
2[a] - 2	-		-		-		-		-		-		1	(.89)			1	(.82)	1	(.94)
2 - 3	22	(33.30)	1	(.93)	5	(5.39)	16	(18.79)	1	(.98)	20	(20.00)	12	(11.66)	5	(8.06)	82	(99.70)	81	(98.22)
2 - 4[a]	-		-		-		-		-		1	(.86)	-				1	(.82)	1	(.94)
2 - 4	2	(1.76)	-		1	(.90)	-		-		1	(.86)	-				4	(3.46)	4	(3.45)
Rest	-	(.25)	-		-	(.10)	-		-		-	(.24)	-	(.11)			-	(.88)	-	(.74)
Total	279		14		54		164		40		184		137		62		934		894	
No. of classes after pooling	7		2		4		5		1		5		4		3		8		8	
D.F.	3		-		-		2		-		-		-		-		2		2	
X^2	30.34						5.21										25.04		24.13	
Probability	.001 > P						0.10 > 0.05										0.001 > P		0.001 > P	

D.F. equals the number of degrees of freedom possible according to the formula $\frac{1}{2}(m^2 - m)$, where m is the number of alleles, minus the number of phenotype classes lost by pooling.

six samples the numbers of individuals in the different phenotype classes were too low for similar analysis.

The allele frequencies in all eight samples showed considerable similarity although the frequency of allele 2 was noticeably higher in sample 12, from Iceland, see Table 46. Despite the significant deviation from genetic equilibrium in sample 4 the allele frequencies of this sample did not differ from those of the remainder. A contingency test was carried out on allele distribution between the samples, made possible by pooling all the alleles except the most common allele 2; when all the samples were included in a test homogeneity was indicated (X^2 = 13.54, D.F. = 7, $0.10 \geq P \geq 0.05$). Despite this result the allele frequencies of the Icelandic sample were very different from all those of the North and Irish Seas' samples, where considerable similarity was present. A contingency test of allele distribution between the Icelandic sample and the pooled North and Irish Sea data showed a significant difference in the distribution (X^2 = 8.91, D.F. = 1, $0.01 \geq P \geq 0.001$) see Table 47.

In an attempt to test the data further a contingency test was made on the phenotype distribution. Unfortunately to obtain large enough values in the expected phenotype distribution to give statistical validity to a X^2 test all phenotype classes, except the most common phenotype 2, were pooled, with a consequent loss of sensitivity. However a test of phenotype distribution throughout the samples did not suggest homogeneity (X^2 = 14.45, D.F. = 7, $0.05 \geq P \geq 0.02$); a significant deviation from the expected distribution was found in the Icelandic sample, number 12, see Table 48. When the test was repeated after removal of this sample

Table 46 G3PDH:- Frequencies of alleles found in eight samples from different localities

Year and Locality	Allele						No. of fish tested
	1	2a	2	3	4[a]	4	
1977							
Gabbard (4)	.0520	-	.8763	.0631	-	.0036	279
White Bank (6)	.0357	-	.9286	.0357	-	-	14
Maughold Head (9)	.0370	-	.8931	.0556	-	.0093	54
Scarborough Gd. (10)	.0793	-	.8537	.0671	-	-	164
1978							
Off Rekjanes II (12)	-	-	.9875	.0125	-	-	40
1976							
Lowestoft (13)	.0625	-	.8696	.0625	.0027	.0027	184
Cleethorpes (14)	.0511	.0036	.8978	.0474	-	-	137
Filey (15)	.0323	-	.8952	.0726	-	-	62

Table 47 A contingency test to examine allele distribution at the G3PDH locus between the Icelandic sample, number 12, and the remainder.

Sample	Allele 2			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
12	79	70.58	1.0045	1	9.42	7.5262	80	8.53	0.01 > 0.001
Rest	1569	1577.42	.0449	219	210.58	.3367	1788	.38	0.70 > 0.50
Totals	1648			220			1868		

Overall X^2 = 8.91

D.F. = 1

0.01 > Probability > 0.001

Table 48 A contingency test to examine phenotype distribution at the G3PDH locus in the eight samples tested.

Sample	Phenotype 2			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
4	219	219.85	.0033	60	59.15	.0122	279	.02	0.90> 0.80
6	12	11.03	.0853	2	2.97	.3168	14	.40	0.70> 0.50
9	44	42.55	.0494	10	11.45	.1836	54	.23	0.70> 0.50
10	120	129.23	.6592	44	34.77	2.4502	164	3.11	0.10> 0.05
12	39	31.52	1.7751	1	8.48	6.5979	40	8.37	0.01> 0.001
13	139	144.99	.2475	45	39.01	.9198	184	1.17	0.30> 0.20
14	112	107.96	.1512	25	29.04	.5620	137	.71	0.50> 0.30
15	51	48.86	.0937	11	13.14	.3485	62	.44	0.70> 0.50
Totals	736			198			934		

Overall X^2 = 14.45

D.F. = 7

0.05> Probability> 0.02.

homogeneity was indicated (X^2 = 5.54, D.F. = 6, 0.50> P> 0.30).

A X^2 test was carried out on the overall observed phenotype distribution, excluding sample 12 from Iceland, and that expected according to Hardy-Weinberg. Significant deviation from genetic equilibrium was found (X^2 = 24.13, D.F. = 2, 0.001> P), particularly due to excesses of phenotype 2 and phenotype 3 individuals and a deficiency of phenotype 2-3 individuals, see Table 45. A similar test on the overall distribution including sample 12 gave a similar result (X^2 = 25.04, D.F. = 2, 0.001> P), again see Table 45.

(b) MDH-A

Not all the samples were tested for this enzyme due to the reasons already explained for G3PDH. Details of the distribution of phenotypes in the eleven samples tested are listed in Table 49; again the numbers in parentheses are those expected according to Hardy-Weinberg. X^2 tests were possible for eight of the 11 samples. In six of the samples the observed phenotype distributions did not differ from those expected assuming genetic equilibrium but in two samples, number 4 from the Gabbard, and number 10 from the Scarborough Ground, both collected in 1977, significant excesses of homozygotes and deficiencies in heterozygotes were observed. However the allele frequencies of these two samples were similar to those found in the other nine samples, see Table 50.

Following the statistical procedure established for data at the G3PDH locus a contingency test of allele distribution throughout the samples was carried out, although only made possible by pooling alleles 1 and 3. This test indicated heterogeneity

Table 49 MDH-A:- The numbers of types are shown for each of the eleven samples. The expected numbers in parentheses assume perfect genetic balance at each position sampled.

Phenotypes	Year and Locality											
	1975			1977			1976				Overall distribution	Overall distribution - samples 9 and 10
	Gabbard (1)	West Mud Hole (2)	Castle Ground (3)	Gabbard (4)	White Bank (6)	Moray Firth (8)	Maughold Hd. (9)	Scarboro' Gd. (1)	Lowestoft (13)	Cleethorpes (14)	Filey (15)		
1 - 1	- (.02)	-	-	1 (.02)	-	-	-	- (.03)	-	- (.03)	-	1 (.06)	1 (.04)
2 - 2	204 (204.48)	198 (195.36)	205 (205.75)	223 (219.56)	10 (10.28)	56 (53.83)	37 (35.05)	121 (117.09)	152 (152.56)	105 (103.95)	50 (49.69)	1361 (1346.52)	1203 (1195.16)
3 - 3	2 (2.10)	6 (3.14)	2 (2.74)	5 (3.01)	- (.29)	3 (.83)	4 (2.04)	7 (3.35)	1 (1.56)	3 (2.41)	1 (.68)	34 (21.36)	23 (16.55)
1 - 2	4 (3.60)	2 (1.77)	-	2 (4.46)	-	-	-	4 (4.23)	-	3 (3.43)	-	15 (17.69)	11 (13.35)
1 - 3	- (.37)	- (.22)	-	1 (.52)	-	-	-	1 (.71)	-	1 (.52)	-	3 (2.23)	2 (1.57)
2 - 3	42 (41.44)	44 (49.50)	49 (47.51)	47 (51.43)	4 (3.43)	9 (13.35)	13 (16.91)	32 (39.59)	32 (30.88)	30 (31.66)	11 (11.63)	313 (339.15)	268 (281.32)
Total	252	250	256	279	14	68	54	165	185	142	62	1727	1508
No. of classes after pooling	4	4	3	4	2	2	3	4	3	4	2	5	5
D.F.	1	1	1	1	-	-	1	1	1	1	-	2	2
X^2	0.15	3.25	0.25	5.15			2.90	5.34	0.24	0.52		11.49	4.81
Probability	0.70>0.50	0.10>0.05	0.70>0.50	0.05>0.02			0.10>0.05	0.05>0.02	0.70>0.50	0.50>0.30		0.01>0.001	0.10>0.05

Table 50 MDH-A :- Frequencies of alleles found in eleven samples separated by time and location

Year and Locality	Allele 1	2	3	No. of fish tested
1975				
Gabbard (1)	.0079	.9008	.0913	252
West Mud Hole (2)	.0040	.8840	.1120	250
Castle Ground (3)	-	.8965	.1035	256
1977				
Gabbard (4)	.0090	.8871	.1039	279
White Bank (6)	-	.8571	.1429	14
Moray Firth (8)	-	.8897	.1103	68
Maughold Head (9)	-	.8056	.1944	54
Scarboro' Ground (10)	.0152	.8424	.1424	165
1976				
Lowestoft (13)	-	.9081	.0919	185
Cleethorpes (14)	.0141	.8556	.1303	142
Filey (15)	-	.8952	.1048	62

(X^2 = 18.86, D.F. = 10, 0.05> P> 0.02) due to significant excesses of alleles 1 and 3 in samples 9 and 10, from Maughold Head and the Scarborough Ground respectively, see Table 51. The reason for this heterogeneity is not obvious. Sample 9 was the only one collected from an Irish Sea Spawning ground and might have been expected to be genetically distinct from the other samples due to its geographical separation, although no such differences were observed at any other locus. Sample 10 was from the Flamborough Off spawning ground, an area also represented by four other samples, numbers 3, 13, 14 and 15, all of which complied with the hypothesis of homogeneity between samples. A contingency test of allele distribution between samples without the inclusion of samples 9 and 10 indicated homogeneity (X^2 = 16.07, D.F. = 8, 0.70> P> 0.50).

A similar test of phenotype distribution throughout the samples was carried out. This suggested homogeneity (X^2 = 11.91, D.F. = 10, 0.30> P> 0 .20) though unfortunately this test was made possible only by pooling all phenotypes except the most common phenotype 2 homozygote.

A X^2 test on the overall observed phenotype distribution and that expected assuming genetic equilibrium gave a significant result (X^2 = 11.14, D.F. = 2, 0.01> P> 0.001), due to excesses of phenotype 2 and phenotype 3 individuals and a deficiency of phenotype 2-3 individuals, see Table 49. Retesting this distribution after the removal of samples 9 and 10 indicated genetic equilibrium (X^2 = 4.81, D.F. = 2, 0.10> P> 0.05), again see Table 49; although excesses of homozygotes were still present these were not significant.

Table 51 A contingency test to examine allele distribution at the MDH-A locus in the eleven samples tested.

Sample	Allele 2			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	454	445.05	.1800	50	58.95	1.3588	504	1.54	0.30> 0.20
2	442	441.52	.0005	58	58.48	.0039	500		
3	459	452.11	.1050	53	59.89	.7927	512	.90	0.50> 0.30
4	495	492.73	.0105	63	65.27	.0789	558	.09	0.80> 0.70
6	24	24.72	.0210	4	3.28	.1580	28	.18	0.70> 0.50
8	121	120.09	.0069	15	15.91	.0520	136	.06	0.90> 0.80
9	87	95.37	.7346	21	12.63	5.5469	108	6.28	0.02> 0.01
10	278	291.40	.6162	52	38.60	4.6518	330	5.27	0.05> 0.02
13	336	326.72	.2693	34	43.28	2.0329	370	2.30	0.20> 0.10
14	243	250.78	.2414	41	33.22	1.8220	284	2.06	0.20> 0.10
15	111	109.50	.0205	13	14.50	.1522	124	.18	0.70> 0.50
Totals	3050			404			3454		

Overall X^2 = 18.86
D.F. = 10
0.05> Probability> 0.02

(c) PGM

All 15 samples were tested for this enzyme and details of their phenotype distributions are listed in Table 52; once again the numbers of different types according to Hardy-Weinberg expectations are given in parentheses. As before X^2 values were obtained from pooled data where necessary; in three samples the numbers were too low to be tested. In only one sample, number 10, collected from the Scarborough Ground in 1977, did the observed phenotype distribution differ significantly from the expected, when an excess of phenotype 3 and phenotype 4 individuals and a deficit of phenotype 3-4 individuals was found.

The allele frequencies for sample 10 did not differ particularly from those of the other North Sea samples, see Table 53, but the two samples from Icelandic waters, numbers 11 and 12, had distinctly lower values for alleles 2 and 3 than any of the other samples. These frequency differences were reflected in a contingency test of allele distribution throughout all the samples, made possible only by pooling all the alleles except the most common allele 4; heterogeneity was indicated (X^2 = 63.60, D.F. = 14, $0.001 > P$), see Table 54. This result was mainly due to large deviations from expected within the two Icelandic samples but with signficiant contributions from samples 5 and 8 from the North Sea. When the two Icelandic samples were removed and the allele distribution between the remaining 13 samples tested no evidence of heterogeneity was found (X^2 = 15.30, D.F. = 12, $0.30 > P > 0.20$).

A similar contingency test on phenotype distribution throughout all the samples was carried out, made possible only by pooling all

s of types are shown for each of the fifteen samples. The expected numbers in parentheses assume alance at each position sampled.

ocality	1977					1978						1976			Combined distribution of samples 11 & 12	Overall distribution minus 11 & 12
	West Mud Hole (2)	Castle Ground (3)	Gabbard (4)	West Mud Hole (5)	White Bank (6)	N.E. Cleaver Bank (7)	Moray Firth (8)	Maughold Hd. (9)	Scarboro' Gd. (10)	Off Rekjanes I (11)	Off Rekjanes II (12)	Lowestoft (13)	Cleethorpes (14)	Filey (15)		
	- (.02)	- (.00)	- (.00)	-	-	(.00) -	-	-	- (.00)	1 (1.69)	- (.39)	- (.05)	- (.01)	-	1 (2.07)	- (.05)
	30 (30.98)	38(37.91)	47 (41.03)	22 (14.67)	19 (13.93)	11 (9.22)	7 (4.76)	15 (14.72)	34 (25.11)	61 (52.28)	8 (7.23)	24 (22.13)	17 (15.23)	6 (7.46)	69 (59.51)	303 (269.78)
	92 (95.48)	91(90.27)	103 (98.18)	66 (59.34)	30 (27.18)	37(34.90)	37 (35.31)	38 (38.05)	92 (86.09)	75 (64.92)	8 (9.03)	71 (72.73)	62 (58.97)	24 (24.53)	83 (73.93)	835 (811.53)
	2 (3.17)	4 (2.70)	3 (3.83)	- (.33)	1 (.82)	- (.34)	(.27)	- (.74)	2 (3.39)	6 (4.26)	-	- (1.04)	2 (.99)	1 (.69)	6 (4.27)	16 (19.96)
	7 (5.56)	3 (4.17)	7 (5.93)	1 (.66)	1 (1.14)	1 (.65)	1 (.73)	2 (1.19)	8 (6.27)	4 (4.75)	-	3 (1.88)	1 (1.94)	1 (1.26)	4 (4.76)	40 (34.61)
	1 (1.41)	1 (.77)	1 (.77)	-	-	1 (.34)	-	-	- (.34)	20 (18.79)	2 (3.40)	- (2.07)	1 (.65)	-	22 (22.18)	6 (7.56)
	3 (2.47)	1 (1.19)	1 (1.19)	-	-	- (.65)	-	-	1 (.63)	22 (20.94)	6 (3.80)	6 (3.76)	1 (1.28)	-	28 (24.72)	15 (13.11)
	113(108.77)	116(116.99)	116(126.94)	45 (59.00)	33 (38.91)	32(35.89)	22 (25.94)	47 (47.32)	78 (92.99)	96 (116.51)	16 (16.15)	80 (80.25)	55 (59.94)	29 (27.05)	112 (132.66)	878 (935.82)
	- (.70)	- (.77)	- (.39)	- (.33)	- (.41)	-	- (.27)	2 (1.49)	- (1.02)	1 (.86)	-	- (.35)	1 (.99)	1 (.35)	1 (.86)	6 (8.92)
	2 (1.24)	2 (1.19)	1 (.60)	1 (.66)	1 (.57)	-	1 (.73)	2 (2.40)	3 (1.89)	1 (.96)	-	1 (.63)	2 (1.94)	- (.63)	1 (.96)	19 (15.47)
	(.10)	(.04)	(.14)	(.01)	(.04)	(.01)	(.00)	(.09)	(.27)	(1.04)	(.00	(.11)	(.06)	(.03)	- (1.08)	- (1.18)
	250	256	279	135	83	82	68	106	218	287	40	185	142	62	327	2118
	8	8	7	4	5	4	4	6	8	9	5	8	7	5	9	10
	3	3	2	-	1	-	-	2	3	4	2	3	2	1	4	5
	1.36	1.76	2.46		1.90			0.25	8.74	8.13	2.32	5.33	1.91	0.54	7.93	13.55
	0.80>0.70	0.70>0.50	0.30>0.20		0.20>0.10			0.90>0.80	0.05>0.02	0.10>0.05	0.50>0.30	0.20>0.10	0.50>0.30	0.50 > 0.30	0.10>0.05	0.02>0.01

Table 53 PGM:- Frequencies of alleles found in fifteen samples separated by time and location.

Year and Locality	Allele					No. of fish tested
	1	2	3	4	5	
1975						
Gabbard (1)	.0099	.0060	.3690	.6052	.0099	252
West Mud Hole (2)	.0180	.0080	.3520	.6180	.0040	250
Castle Ground (3)	.0137	.0040	.3848	.5938	.0040	256
1977						
Gabbard (4)	.0179	.0036	.3835	.5932	.0018	279
West Mud Hole (5)	.0037	-	.3296	.6630	.0037	135
White Bank (6)	.0120	-	.4096	.5723	.0060	83
N.E. Cleaver Bank (7)	.0061	.0061	.3354	.6524	-	82
Moray Firth (8)	.0074	-	.2647	.7206	.0074	68
Maughold Head (9)	.0094	-	.3726	.5991	.0189	106
Scarborough Ground (10)	.0229	.0023	.3394	.6284	.0069	218
1978						
Off Rekjanes I (11)	.0174	.0767	.4268	.4756	.0035	287
Off Rekjanes II (12)	-	.1000	.4250	.4750	-	40
1976						
Lowestoft (13)	.0081	.0162	.3459	.6270	.0027	185
Cleethorpes (14)	.0106	.0070	.3275	.6444	.0106	142
Filey (15)	.0161	-	.3468	.6290	.0081	62

Table 54 A contingency test to examine allele distribution at the PGM locus in the fifteen samples tested.

Sample	Allele 4			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	305	302.30	.0241	199	201.70	.0361	504	.0602	0.90> 0.80
2	309	299.90	.2761	191	200.10	.4138	500	.6899	0.50> 0.30
3	304	307.10	.0313	208	204.90	.0469	512	.0782	0.80> 0.70
4	331	334.69	.0407	227	223.31	.0610	558	.1017	0.80> 0.70
5	179	161.94	1.7972	91	108.06	2.6934	270	4.4906	0.50> 0.02
6	95	99.57	.2098	71	66.43	.3144	166	.5242	0.50> 0.30
7	107	98.37	.7571	57	65.63	1.1348	164	1.8919	0.20> 0.10
8	98	81.57	3.3094	38	54.43	4.9595	136	8.2689	0.01> 0.001
9	127	127.16	.0002	85	84.84	.0003	212	.0005	0.99> 0.98
10	274	261.51	.5965	162	174.49	.8940	436	1.4905	0.30> 0.20
11	273	344.28	14.7579	301	229.72	23.1175	574	36.8754	0.001> P
12	38	47.98	2.0759	42	32.02	3.1106	80	5.1865	0.05> 0.02
13	232	221.92	.4579	138	148.08	.6862	370	1.1441	0.30> 0.20
14	183	170.34	.9409	101	113.66	1.4101	284	2.3510	0.20> 0.10
15	78	74.37	.1772	46	49.63	.2655	124	.4426	0.70> 0.50
Totals	2933			1957			4890		

Overall X^2 = 63.60
D.F. = 14
0.001 > Probability

the phenotype classes except the homozygous 4 and the heterozygous 3-4 classes. Signficiant heterogeneity was found (X^2 = 102.05, D.F. = 28, 0.001>P), see Table 55. Four samples deviated significantly from the excepted distribution but again the two outstanding differences were present in the two Icelandic samples, numbers 11 and 12. When these two geographically remote samples were omitted and the analysis repeated homogeneity was found between the remaining samples from the North and Irish Seas (X^2 = 26.22, D.F. = 24, 0.50>P>0.30).

Comparisons of both allele and phenotype distributions between the two Icelandic samples indicated homogeneity (allele distribution X^2 = 0.00, P>0.99; phenotype distribution : X^2 = 0.96, D.F. = 2, 0.70>P>0.50).

A X^2 test following application of the Hardy-Weinberg Principle to the combined phenotype distribution of these two samples suggested genetic equilibrium (X^2 = 7.93, D.F. = 4, 0.10>P>0.05), see Table 52. However when the same analysis was applied to the overall phenotype distribution of the remaining 13 samples a significant deviation from genetic equilibrium was indicated (X^2 = 13.55, D.F. = 5, 0.02>P>0.01), again see Table 52. This was particularly due to the number of phenotype 3 and phenotype 4 individuals observed being greater than expected with a corresponding deficit in the numbers of phenotype 3-4 individuals observed. Although the X^2 was not significant this pattern was also shown in the phenotype distribution of the combined Icelandic samples.

(d) GPI

(i) GPI-A:- Thirteen samples were tested for this enzyme and details of their phenotype distributions are given in Table 56;

Table 55 A contingency test to examine phenotype distribution at the PGM locus in the fifteen samples tested.

Sample	Phenotype 4			Phenotype 3-4			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	92	94.65	.0742	112	102.08	.9640	48	55.27	.9563	252	1.99	0.50 > 0.30
2	92	93.90	.0384	113	101.27	1.3587	45	54.83	1.7623	250	3.16	0.30 > 0.20
3	91	96.16	.0028	116	103.70	1.4589	49	56.14	.9081	256	2.37	0.50 > 0.30
4	103	104.80	.0309	116	113.02	.0786	60	61.19	.0231	279	.13	0.95 > 0.90
5	66	50.71	4.6102	45	54.68	1.7137	24	29.61	1.0629	135	7.39	0.05 > 0.02
6	30	31.18	.0447	33	33.62	.0114	20	18.20	.1780	83	.23	0.90 > 0.80
7	37	30.80	1.2481	32	33.22	.0448	13	17.98	1.3793	82	2.67	0.30 > 0.20
8	37	25.54	5.1422	22	27.55	1.1181	9	14.91	2.3426	68	8.60	0.02 > 0.01
9	38	39.82	.0832	47	42.94	.3839	21	23.25	.2177	106	.68	0.80 > 0.70
10	92	81.88	1.2508	78	88.31	1.2037	48	47.81	.0008	218	2.46	0.30 > 0.20
11	75	107.43	9.7897	96	115.85	3.4011	116	62.72	43.5778	287	56.77	0.001 > P
12	8	15.02	3.2810	16	16.20	.0025	16	8.77	5.9604	40	9.24	0.01 > 0.001
13	71	69.49	.0328	80	74.93	.3431	34	40.57	1.0640	185	1.44	0.50 > 0.30
14	62	53.34	1.4060	55	57.52	.1104	25	31.14	1.2106	142	2.73	0.30 > 0.20
15	24	23.29	.0216	29	25.11	.6026	9	13.60	1.5559	62	2.18	0.50 > 0.30
Totals	918			990			537			2445		

Overall X^2 = 102.05
D.F. = 28
0.001 > Probability

as before the numbers of different types expected according to Hardy-Weinberg are given in parentheses. X^2 tests were only possible for two of the samples, numbers 1 and 2, where agreement between observed and expected distributions suggested genetic equilibrium. In the remaining 11 samples the expected numbers in the different phenotype classes were too low for statistical analysis. However the allele frequencies of all the samples were apparently similar, see Table 57.

Despite this a contingency test of allele distribution throughout the samples, only made possible by pooling all the alleles except the most common allele, allele 2, indicated heterogeneity (X^2 = 22.65, D.F. = 12, $0.05 > P > 0.02$), see Table 58. This was due to sample 1, collected from the Gabbard in 1975, where a deficiency of allele 2 and an excess of rarer alleles was found. A repeat of the test after the removal of this sample showed homogeneity between the remaining samples (X^2 = 14.86, D.F. = 11, $0.20 > P > 0.10$). A similar analysis of phenotype distribution throughout all the samples, only made possible by pooling all the phenotype classes except the most common homozygote, phenotype 2, also indicated heterogeneity (X^2 = 21.70, D.F. = 12, $0.05 > P > 0.02$), see Table 59. This was again due to sample 1, where a deficiency of phenotype 2 individuals and an excess of other types was present. When this sample was omitted and thetest repeated homogeneity was found (X^2 = 13.86, D.F. = 11, $0.30 > P > 0.20$).

As no differences between the Icelandic and the remaining samples are apparent from the results it does not seem reasonable to attempt to differentiate between these two groups of samples

Table 56 GPI-A:- The numbers of expected types are shown for each of the thirteen samples. The expected numbers in parentheses assume perfect genetic balance at each position sampled.

Phenotypes	Year and Locality																																			
	1975						1977								1978								1976						Overall distribution		Overall distribution - samples 11 and 12		Overall distribution - sample 1	
	Gabbard (1)		West Mud Hole (2)		Castle Ground (3)		Gabbard (4)		White Bank (6)		Moray Firth (8)		Maughold Head (9)		Scarboro' Gd. (10)		Off Rekjanes I (11)		Off Rekjanes II (12)		Lowestoft (13)		Cleethorpes (14)		Filey (15)									
1 - 1	-	(.62)	1	(.48)	-	(.22)	-	(.20)	-		-	(.03)	-	(.02)	1	(.15)	-	(.42)	-	(.06)	2	(.49)	1	(.21)	-		5	(2.63)	5	(2.15)	5	(2.06)
2 - 2	211	(210.83)	217	(216.23)	229	(227.84)	245	(246.05)	14	(14)	64	(64.06)	51	(51.04)	149	(148.44)	262	(262.52)	37	(37.06)	162	(160.83)	130	(129.29)	60	(60.02)	1831	(1827.68)	1532	(1527.93)	1620	(1617.01)
3 - 3	-	(.22)	1	(.17)	1	(.14)	-	(.07)	-		-	(.00)	-		-	(.02)	-	(.00)	-		-		-	(.00)		(.00)	2	(.40)	2	(.45)	2	(.24)
1[a] - 2	3	(2.74)	-		2	(1.88)	9	(8.44)	-		-		1	(.98)	3	(2.85)	2	(1.92)	-		3	(2.79)	1	(.95)	1	(.99)	25	(23.64)	23	(21.77)	22	(20.84)
1 - 2	23	(22.87)	20	(20.46)	14	(14.15)	15	(14.10)	-		3	(2.92)	2	(1.94)	8	(9.48)	22	(21.03)	3	(2.89)	15	(17.73)	9	(10.49)	-		134	(138.78)	109	(114.68)	111	(115.57)
1 - 3	2	(.74)	-	(.57)	1	(.35)	-	(.24)	-		-	(.02)	-		-	(.12)	-	(.04)	-		-		-	(.04)	-		3	(2.04)	3	(1.98)	1	(1.42)
2 - 3	13	(13.72)	11	(12.09)	9	(11.30)	9	(8.44)	-		1	(.98)	-		4	(3.79)	1	(.93)	-		-		1	(.95)	1	(.99)	50	(53.86)	49	(52.63)	37	(39.79)
2 - 4	-		-		-		1	(.94)	-		-		-		-		-		-		3	(2.79)	-		-		4	(3.88)	4	(3.90)	4	(3.79)
Rest	-	(.26)	-	-	-	(.12)	-	(.52)	-		-		-	(.02)	-	(.15)	-	(.14)	-		-	(.37)	-	(.07)	-	(.01)	-	(1.14)	-	(1.51)		(1.52)
Total	252		250		256		279		14		68		54		165		287		40		185		142		62		2054		1727		1802	
No. of classes after pooling	5		4		4		5		-		3		3		4		4		2		4		3		2		8		8		8	
D.F.	1		1		-		-		-		-		-		-		-		-		-		-		-		3		3		3	
x^2	0.08		0.61		-																						3.25		4.92		4.93	
Probability	0.80>0.70		0.50>0.30																								0.50>0.30		0.20>0.10		0.20>0.10	

Table 57 GPI-A:- Frequencies of alleles found in thirteen samples separated by time and location

Year and Locality	1[a]	1	2	3	4	No. of fish tested
1975						
Gabbard (1)	.0060	.0496	.9147	.0298	-	252
West Mud Hole (2)	-	.0440	.9300	.0260	-	250
Castle Ground (3)	.0039	.0293	.9434	.0234	-	256
1977						
Gabbard (4)	.0161	.0269	.9391	.0161	.0018	279
White Bank (6)	-	-	1.0000	-	-	14
Moray Firth (8)	-	.0221	.9706	.0074	-	68
Maughold Head (9)	.0093	.0185	.9722	-	-	54
Scarborough Ground (10)	.0091	.0303	.9485	.0121	-	165
1978						
Off Rekjanes I (11)	.0035	.0383	.9564	.0017	-	287
Off Rekjanes II (12)	-	.0375	.9625	-	-	40
1976						
Lowestoft (13)	.0081	.0514	.9324	-	.0081	185
Cleethorpes (14)	.0035	.0387	.9542	.0035	-	142
Filey (15)	.0081	-	.9839	.0081	-	62

Table 58 A contingency test to examine allele distribution at the GPI-A locus in the thirteen samplestested.

Sample	Allele 2			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	461	475.41	.4363	43	28.59	7.2630	504	7.70	0.01 > 0.001
2	465	471.64	.0935	35	28.36	1.5546	500	1.65	0.20 > 0.10
3	483	482.96	.0000	29	29.04	.0001	512	.00	P > 0.99
4	524	526.35	.0105	34	31.65	.1745	558	.20	0.70 > 0.50
6	28	26.41	.0957	-	1.59	1.5900	28	1.69	0.20 > 0.10
8	132	128.29	.1073	4	7.71	1.7852	136	1.89	0.20 > 0.10
9	105	101.87	.0962	3	6.13	1.5982	108	1.69	0.20 > 0.10
10	313	311.28	.0095	17	18.72	.1580	330	.17	0.70 > 0.50
11	549	541.44	.1056	25	32.56	1.7553	574	1.86	0.20 > 0.10
12	77	75.46	.0314	3	4.54	.5224	80	.55	0.50 > 0.30
13	345	349.01	.0461	25	20.99	..7661	370	.81	0.50 > 0.30
14	271	267.89	.0361	13	16.11	.6004	284	.64	0.50 > 0.30
15	122	116.97	.2163	2	7.03	3.5990	124	3.82	0.10 > 0.05
Totals	3875			233			4108		

Overall X^2 = 22.65
D.F. = 12
0.05 > Probability > 0.02

Table 59 A contingency test to examine phenotype distribution at the GPI-A locus in the thirteen samples tested

Sample	Phenotype 2			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1.	211	224.64	.8282	41	27.36	6.8001	252	7.63	0.01> 0.001
2	217	222.86	.1541	33	27.14	1.2653	250	1.42	0.30> 0.20
3	229	228.21	.0027	27	27.79	.0225	256	.03	0.90> 0.80
4	245	248.71	.0553	34	30.29	.4544	279	.51	0.50> 0.30
6	14	12.48	.1851	-	1.52	1.5200	14	1.71	0.20> 0.10
8	64	60.62	.1855	4	7.38	1.5480	68	1.74	0.20> 0.10
9	51	48.14	.1699	3	5.86	1.3958	54	1.57	0.30> 0.20
10	149	147.09	.0248	16	17.91	.2037	165	.23	0.70> 0.50
11	262	255.84	.1483	25	31.16	1.2178	287	1.37	0.30> 0.20
12	37	35.66	.0504	3	4.34	.4137	40	.46	0.50> 0.30
13	162	164.91	.0513	23	20.09	.4215	185	.47	0.50> 0.30
14	130	126.58	.0924	12	15.42	.7585	142	.85	0.50> 0.30
15	60	55.27	.4048	2	6.73	3.3244	62	3.73	0.10> 0.05
	1831			223			2054		

Overall X^2 = 21.70

D.F. = 12

0.05 > Probability > 0.02

at this locus. In fact a X^2 test following application of Hardy-Weinberg to the overall phenotype distribution, minus sample 1, showed genetic equilibrium (X^2 = 4.93, D.F. = 3, 0.20> P> 0.10). A similar test on all the North and Irish Seas data gave the same result (X^2 = 4.92, D.F. = 3, 0.20> P> 0.10); a result repeated when all the available data were analysed (X^2 = 3.25, D.F. = 3, 0.50> P> 0.30). Table 56 gives details of these distributions.

(ii) GPI-B:- The same 13 samples were tested for this enzyme as for GPI-B. Table 60 gives details of the observed phenotype distributions and these expected after application of the Hardy-Weinberg Principle. X^2 tests were only possible for four of the samples, numbers 1, 2, 3 and 11, and in all instances agreement between observed and expected numbers of phenotypes suggested genetic equilibrium. The pattern of allele frequencies was similar throughout all the samples, with the most common allele, allele 6, always forming almost all the gene pool, see Table 61. A contingency test of allele distribution throughout the samples only made possible by pooling all alleles except allele 6, indicated heterogeneity (X^2 = 30.43, D.F. = 12, 0.01> P> 0.001), see Table 62. Significant deficiencies of allele 6 and excesses in samples 2 and 11, collected from the West Mud Hole in 1975 and South West Iceland in 1978 respectively, and a significant excess of allele 6 and a deficiency of other alleles in sample 14, from Cleethorpes beach in 1976, contributed to this result.

A similar test of phenotype distribution throughout all the samples, made possible by pooling all the phenotype classes except the most common phenotype 6, also showed significant

Table 60 GPI-B:- The numbers of types are shown for each of the thirteen samples. The expected numbers in parentheses assume perfect genetic balance at each position sampled.

Phenotypes	Year and Locality												
	1975			1977									
	Gabbard(1)	West Mud Hole(2)	Castle Ground(3)	Gabbard(4)	White Bank(6)	Morex Firth(8)	Maughold Hd.(9)	Scarboro' Gd.(10)	Off Rekjanes I(11)	Off Rekjanes II(12)	Lowestoft(13)	Cleethorpes(14)	Filey(15)
4 - 4	- (.72)	1 (1.09)	- (.56)	- (.47)	- (.02)	- (.01)	- (.04)	- (.22)	1 (2.45)	- (.16)	1 (.19)	- (.06)	- (.02)
6 - 6	213 (211.75)	198 (198.92)	217 (216.64)	240 (240.43)	13 (13.02)	60 (59.29)	48 (48.16)	148 (146.54)	225 (227.43)	34 (34.23)	168 (166.47)	132 (132.18)	56 (55.19)
7 - 7	- (.14)	- (.32)	- (.22)	1 (0.15)	-	1 (.13)	- (.04)	- (.04)	- (.00)	-	- (.05)	- (.02)	- (.02)
1 - 6	-	-	-	1 (0.93)	-	-	-	-	-	-	-	-	-
2 - 4	- (.16)	- (.20)	- (.09)	- (.04)	-	- (.01)	-	- (.04)	-	- (.06)	- (.03)	-	1 (.02)
2 - 6	3 (2.75)	3 (2.68)	2 (1.84)	1 (.93)	-	1 (.94)	-	1 (.93)	-	1 (.92)	1 (.95)	-	- (.95)
4 - 6	24 (24.75)	30 (29.44)	22 (22.08)	23 (21.34)	1 (.96)	2 (1.87)	3 (2.84)	11 (11.32)	51 (47.16)	5 (4.62)	9 (11.37)	6 (5.78)	1 (1.88)
4 - 7	3 (.64)	1 (1.19)	2 (.70)	- (.54)	-	- (.09)	- (.08)	1 (.18)	- (.19)	-	1 (.19)	- (.06)	- (.03)
5[a] - 6	-	-	-	-	-	-	-	-	8 (7.10)	-	-	-	1 (.95)
5 - 6	-	-	-	1 (.93)	-	-	-	1 (.93)	-	-	-	-	-
6 - 7[a]	-	-	-	-	-	-	-	-	-	-	-	1 (.96)	1 (.95)
6 - 7	9 (11.00)	17 (16.06)	13 (13.80)	11 (12.07)	-	4 (5.60)	3 (2.84)	2 (4.73)	2 (1.79)	-	5 (5.69)	3 (2.90)	2 (1.88)
6 - 8	-	-	-	1 (.93)	-	-	-	-	-	-	-	-	-
Rest	(.09)	(.10)	(.07)	(.24)	-	(.06)	-	(.07)	(.88)	(.01)	(.06)	(.04)	(.11)
Total	252	250	256	279	14	68	54	165	287	40	185	142	62
No. of classes after pooling	5	6	5	4	1	4	3	4	6	3	4	4	4
D.F.	1	2	1						2				
X^2	1.31	2.68	0.14						2.41				
Probability	0.30>0.20	0.30>0.20	0.80>0.70						0.30>0.20				

Table 61 GPI-B:- Frequencies of alleles found in thirteen samples separated by time and location.

Year and Locality	Allele 1	2	4	5[a]	5	6	7[a]	7	8	No. of fish tested
1975										
Gabbard (1)	-	.0060	.0536	-	-	.9167	-	.0238	-	252
West Mud Hole (2)	-	.0060	.0660	-	-	.8920	-	.0360	-	250
Castle Ground (3)	-	.0039	.0469	-	-	.9199	-	.0293	-	256
1977										
Gabbard (4)	.0018	.0018	.0412	-	.0018	.9283	-	.0233	.0018	279
White Bank (6)	-	-	.0357	-	-	.9643	-	-	-	14
Moray Firth (8)	-	.0074	.0147	-	-	.9338	-	.0441	-	68
Maughold Head (9)	-	-	.0278	-	-	.9444	-	.0278	-	54
Scarborough Ground (10)	-	.0030	.0364	-	.0030	.9424	-	.0152	-	165
1978										
Off Rekjanes I (11)	-	-	.0923	.0139	-	.8902	-	.0035	-	287
Off Rekjanes II (12)	-	.0125	.0625	-	-	.9250	-	-	-	40
1976										
Lowestoft (13)	-	.0027	.0324	-	-	.9486	-	.0162	-	185
Cleethorpes (14)	-	-	.0211	-	-	.9648	.0035	.0106	-	142
Filey (15)	-	.0081	.0161	.0081	-	.9435	.0081	.0161	-	62

Table 62 A contingency test to examine allele distribution at the GPI-B locus in the thirteen samples tested.

Sample	Allele 6			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	462	465.11	.0208	42	38.89	.2487	504	.27	$0.70 > 0.50$
2	446	461.42	.5153	54	38.58	6.1632	500	6.68	$0.01 > 0.001$
3	471	472.49	.0047	41	39.51	.0562	512	.06	$0.90 > 0.80$
4	518	514.94	.0182	40	43.06	.2175	558	.24	$0.70 > 0.50$
6	27	25.84	.0521	1	2.16	.6230	28	.68	$0.50 > 0.30$
8	127	125.51	.0177	9	10.49	.2116	136	.23	$0.70 > 0.50$
9	102	99.67	.0545	6	8.33	.6517	108	.71	$0.50 > 0.30$
10	311	304.54	.1370	19	25.46	1.6391	330	1.78	$0.20 > 0.10$
11	511	529.71	.6609	63	44.29	7.9039	574	8.56	$0.01 > 0.001$
12	74	73.83	.0004	6	6.17	.0047	80	.01	$0.95 > 0.90$
13	351	341.45	.2671	19	28.55	3.1945	370	3.46	$0.10 > 0.05$
14	274	262.08	.5421	10	21.92	6.4820	284	7.02	$0.01 > 0.001$
15	117	114.43	.0577	7	9.57	.6902	124	.75	$0.50 > 0.30$
Totals	3791			317			4108		

Overall $X^2 = 30.43$

D.F. = 12

$0.01 >$ Probability > 0.001

heterogeneity (X^2 = 35.19, D.F. = 12, 0.001>P), see Table 63. This was due to significant deficiencies of phenotype 6 individuals and excesses of other phenotypes in samples 2 and 11 and significantly opposite distributions in samples 13 and 14; sample 13 was collected from the beach at Lowestoft in 1976.

The Icelandic sample, number 11, represents a geographically remote area and may be expected to be genetically distinct but the phenotype and allele distributions of the other sample from Iceland, number 12, although consisting of fewer individuals, did not differ from the distributions expected assuming homogeneity between all samples, see Tables 62 and 63. However comparisons between these two Icelandic samples showed homogeneity both in allele and phenotype distributions (Allele distribution : X^2 = 0.90, D.F. = 1, 0.50>P>0.30; phenotype distribution : X^2 = 0.93, D.F. = 1, 0.50>P>0.30).

Similar tests between the remaining eleven North and Irish Seas samples still indicated heterogeneity in both distributions (Allele distribution : X^2 = 21.86, D.F. = 10, 0.02>P>0.01; phenotype distribution : X^2 = 24.06, D.F. = 10, 0.01>P>0.001), see Tables 64 and 65. As in previous tests significant deviations from the expected distributions were found in samples 2 and 14.

Sample 2 from the West Mud Hole is the only sample representing the Central North Sea spawning ground and as a consequence may indicate a difference between this and other North Sea grounds with respect to this locus.

The two samples of '0' group fish, numbers 13 and 14, collected from beaches, originate from North Sea spawning grounds represented by other samples, although there are differences in the age of individuals and the year of sampling between these '0' group fish

Table 63 A contingency test to examine phenotype distribution at the GPI-B locus in the thirteen samples tested.

Sample	Phenotype 6			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	213	214.95	.0177	39	37.05	.1026	252	.12	0.80> 0.70
2	198	213.24	1.0892	52	36.76	6.3182	250	7.41	0.01> 0.001
3	217	218.36	.0085	39	37.64	.0491	256	.06	0.90> 0.80
4	240	237.98	.0171	39	41.02	.0995	279	.12	0.80> 0.70
6	13	11.94	.0941	1	2.06	.5454	14	.64	0.50> 0.30
8	60	58.00	.0690	8	10.00	.4000	68	.47	0.50> 0.30
9	48	46.06	.0817	6	7.94	.4740	54	.56	0.50> 0.30
10	148	140.74	.3745	17	24.26	2.1726	165	2.55	0.20> 0.10
11	225	244.80	1.6015	62	42.20	9.2900	287	10.89	0.001> P
12	34	34.12	.0004	6	5.88	.0024	40	.003	0.98> 0.95
13	168	157.80	.6593	17	27.20	3.8250	185	4.48	0.05> 0.02
14	132	121.12	.9773	10	20.88	5.6693	142	6.65	0.01> 0.001
15	56	52.88	.1841	6	9.12	1.0676	62	1.25	0.30> 0.20
Totals	1752			302			2054		

Overall X^2 = 35.19
D.F. = 12
0.001 > Probability

Table 64 A contingency test to examine allele distribution at the GPI-B locus in the eleven samples from the North and Irish Seas.

Sample	Allele 6			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	462	467.81	.0722	42	36.19	.9327	504	1.00	$0.50 > 0.30$
2	446	464.10	.7059	54	35.90	9.1256	500	9.83	$0.01 > 0.001$
3	471	475.24	.0378	41	36.76	.4891	512	.53	$0.50 > 0.30$
4	518	517.94	.0000	40	40.06	.0000	558	.00	$0.99 < P$
6	27	25.99	.0392	1	2.01	.5075	28	.55	$0.50 > 0.30$
8	127	126.24	.0046	9	9.76	.0592	136	.06	$0.90 > 0.80$
9	102	100.25	.0305	6	7.75	.3952	108	.43	$0.70 > 0.50$
10	311	306.31	.0718	19	23.69	.9285	330	1.00	$0.50 > 0.30$
13	351	343.43	.1669	19	26.67	2.1568	370	2.32	$0.20 > 0.10$
14	274	263.61	.4095	10	20.39	5.2944	284	5.70	$0.02 > 0.01$
15	117	115.10	.0314	7	8.90	.4056	124	.44	$0.70 > 0.50$
Totals	3206			248			3454		

Overall $X^2 = 21.86$
D.F. = 10
$0.02 >$ Probability > 0.01

Table 65 A contingency test to examine phenotype distribution at the GPI-B locus in the eleven samples from the North and Irish Seas.

Sample	Phenotype 6			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	213	217.86	.1084	39	34.14	.6918	252	.80	0.50> 0.30
2	198	216.13	1.5208	52	33.87	9.7047	250	11.23	0.001> P
3	217	221.31	.0839	39	34.69	.5355	256	.62	0.50> 0.30
4	240	241.20	.0060	39	37.80	.0381	279	.04	0.90> 0.80
6	13	12.10	.0069	1	1.90	.4263	14	.49	0.50> 0.30
8	60	58.79	.0249	8	9.21	.1590	68	.18	0.70> 0.50
9	48	46.68	.0373	6	7.32	.2380	54	.28	0.70> 0.50
10	148	142.64	.2014	17	22.36	1.2849	165	1.49	0.30> 0.20
13	168	159.93	.4076	17	25.07	2.5977	185	3.01	0.10> 0.05
14	132	122.76	.6955	10	19.24	4.4375	142	5.13	0.05> 0.02
15	56	53.60	.1075	6	8.40	.6857	62	.79	0.50> 0.30
Totals	1493			234			1727		

Overall X^2 = 24.06
D.F. = 10
0.01 > Probability > 0.001

and hatchery reared larvae. However as no evidence of differences between years in samples from similar areas has been found in this study a selection effect with age may be present at this locus. Indeed all three '0' group samples had an excess of phenotype 6 individuals and a deficiency of other types, although these deviations were only significant in samples 13 and 14, see Table 63. Similarly excesses of allele 6 and deficiencies of other alleles were observed in these three samples, although only significantly in sample 14, see Table 62.

A X^2 test between the overall observed phenotype distribution and that expected according to the Hardy-Weinberg Principle was not carried out because of the presence of heterogeneity between samples due not only to differences between Icelandic samples and the remainder but also to differences between the North Sea samples.

(e) 6PGD

Twelve samples were tested for this enzyme and, as for previous enzymes, details of observed and expected phenotype distributions are given, see Table 66. In all the samples the numbers of expected phenotypes in the rarer classes were so low that, after pooling to obtain statistical validity, X^2 tests between observed and expected distributions could not be carried out. All the samples had similar gene frequencies, see Table 67, with alleles 1, 3 and 4 occurring infrequently. The numbers of allele other than the common allele, allele 2, were too low to allow a contingency test of allele distribution throughout all the samples to be carried out. Similarly phenotype distribution could not be tested due to the scarcity of any phenotype other than the common homozygote, phenotype 2. However these tests

Table 66 6PGD:- The numbers of types are shown for each of the twelve samples. The expected numbers in parentheses assume perfect genetic balance at each position sampled.

Phenotypes	Year and Locality											
	1975											
	Gabbard (1)		West Mud Hole (2)		Castle Ground (3)		Gabbard (4)		White Bank (6)		Moray Firth (8	
1 - 1	2	(.14)	1	(.05)	-	(.08)	-	(.20)	-		-	(.01)
2 - 2	231	(229.54)	238	(237.17)	243	(243.17)	264	(264.19)	14	(14.00)	67	(67.00)
3 - 3	-	(.12)	-	(.04)	-	(.02)	-		-		-	
1 - 2	8	(11.45)	5	(6.82)	9	(8.77)	15	(14.61)	-		1	(1.00)
2 - 3	11	(10.49)	6	(5.84)	4	(3.90)	-		-		-	
2 - 4	-		-		-		-		-		-	
Rest		(.26)		(.08)		(.06)		-			-	
Total	252		250		256		279		14		68	
No. of classes after pooling	3		3		3		2		-		-	
D.F.	-		-		-		-		-		-	

	1978				1976					
Maughold Hd. (9)		Scarboro' Gd. (10)		Off Rekjanes II (12)		Lowestoft (13)		Cleethorpes (14)		Filey (15)	
-	(.02)	-	(.05)	-		1	(.07)	-	(.01)	-	(.06)
51	(51.04)	159	(159.05)	40	(40.00)	176	(175.14)	139	(139.01)	56	(55.19)
-	(.00)	-		-		-	(.01)	-	(.00)	1	(1.04)
2	(1.94)	6	(5.90)	-		5	(6.80)	2	(1.97)	4	(3.78)
1	(.98)	-		-		2	(1.94)	1	(.98)	1	(2.83)
-		-		-		1	(.97)	-		-	
	(.02)		-	-		-	(.07)	-	(.03)	-	(.10)
54		165		40		185		142		62	
2		2		-		4		3		3	
-		-		-		-		-		-	

Table 67 6PGD:- Frequencies of alleles found in twelve samples separated by time and location.

Year and Locality	Allele				No. of fish tested
	1	2	3	4	
1975					
Gabbard (1)	.0238	.9544	.0218	-	252
West Mud Hole (2)	.0140	.9740	.0120	-	250
Castle Ground (3)	.0176	.9746	.0078	-	256
1977					
Gabbard (4)	.0269	.9731	-	-	279
White Bank (6)	-	1.0000	-	-	14
Moray Firth (8)	.0074	.9926	-	-	68
Maughold Head (9)	.0185	.9722	.0093	-	54
Scarborough Ground (10)	.0182	.9818	-	-	165
1978					
Off Rekjanes II (12)	-	1.0000	-	-	40
1976					
Lowestoft (13)	.0189	.9730	.0054	.0027	185
Cleethorpes (14)	.0070	.9894	.0035	-	142
Filey (15)	.0323	.9435	.0242	-	62

were possible after removal of the very small sample, number 6, from the White Bank. Heterogeneity was found in the allele distribution between the remaining 11 samples (X^2 = 19.09, D.F. = 10, $0.05 \geq P \geq 0.02$), see Table 68, but not, however, due to any significant deviations in the Icelandic sample, number 12, but due to deficiencies of allele 2 and excesses of other alleles in samples 1 and 15 from the North Sea. A contingency test of allele distribution between samples excluding these two samples indicated homogeneity (X^2 = 7.83, D.F. = 8, $0.50 \geq P \geq 0.80$). A similar test of phenotype distribution between all the samples indicated homogeneity (X^2 = 15.68, D.F. = 10, $0.20 \geq P \geq 0.10$).

The low level of polymorphism made further analysis impossible. Although the allele distributions suggest that differences may exist between areas with respect to this locus both samples 1 and 15 originate from the same spawning grounds as other samples collected in different years; in these samples no evidence of heterogeneity was present.

(f) IDH

Six samples were tested for this enzyme and Table 69 gives details of observed and expected phenotype distributions. The level of polymorphism was consistently low and statistical comparisons between observed and expected numbers of different phenotypes were not possible. The allele frequencies of all the samples were similar, see Table 70, with the most common allele, allele 2, always having a frequency of at least .96. Contingency tests on allele and phenotype distributions throughout the samples both suggested homogeneity (Allele distribution : X^2 = 5.38, D.F. = 5, $0.50 \geq P \geq 0.30$; phenotype distribution : X^2 = 5.46, D.F. = 5, $0.50 \geq P \geq 0.30$).

Table 68 A contingency test to examine allele distribution at the 6PGD locus in all the samples tested except Sample 6.

Sample	Allele 2			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
1	481	490.49	.1836	23	13.51	6.6662	504	6.85	0.01> 0.001
2	487	486.59	.0003	13	13.41	.0125	500	.01	0.95> 0.90
3	499	498.27	.0011	13	13.73	.0388	512	.04	0.90> 0.80
4	543	543.04	.0000	15	14.96	.0001	558	.00	P > 0.99
8	135	132.35	.0531	1	3.65	1.9240	136	1.98	0.20> 0.10
9	105	105.10	.0001	3	2.90	.0034	108	.00	P > 0.99
10	324	321.15	.0253	6	8.85	.9178	330	.94	0.50> 0.30
12	80	77.86	.0588	-	2.14	2.1400	80	2.20	0.20> 0.10
13	360	360.08	.0000	10	9.92	.0006	370	.00	P > 0.99
14	281	276.39	.0769	3	7.61	2.7927	284	2.87	0.10> 0.05
15	117	120.68	.1122	7	3.32	4.0790	124	4.19	0.05> 0.02
Totals	3412			94			3506		

Overall X^2 = 19.09
D.F. = 10
0.05 > Probability > 0.02

Table 69 IDH:- The numbers of types are shown for each of the six samples. The expected numbers in parentheses assume perfect genetic balance at each position sampled.

Phenotypes	Year and Locality													
	1977						1976						Overall	
	Gabbard (4)		Maughold Hd. (9)		Scarboro' Gd. (10)		Lowestoft (13)		Cleethorpes (14)		Filey (15)			
2 - 2	273	(273.02)	53	(53.00)	110	(110.01)	182	(182.02)	138	(138.02)	58	(58.06)	814	(814.10)
1 - 2	1	(.99)	-		-		-		1	(.98)	-		2	(1.98)
2 - 3	4	(3.97)	1	(1.00)	3	(2.97)	2	(1.98)	2	(1.96)	4	(3.88)	16	(15.82)
2 - 4	-		-		-		1	(.99)	1	(.98)	-		2	(1.98)
Rest		(.02)		(.00)		(.02)		(.01)		(.06)		(.06)		(.12)
Total	278		54		113		185		142		62		834	

Numbers are too small for statistical analysis.

Table 70 IDH:- Frequencies of alleles found in six samples from different localities.

Year and Locality	Allele				No. of fish tested
	1	2	3	4	
1977					
Gabbard (4)	.0018	.9910	.0072	-	278
Maughold Head (9)	-	.9907	.0093	-	54
Scarborough Ground (10)	-	.9867	.0133	-	113
1976					
Lowestoft (13)	-	.9919	.0054	.0027	185
Cleethorpes (14)	.0035	.9859	.0070	.0035	142
Filey (15)	-	.9677	.0323	-	62

Due to the low level of polymorphism a X^2 test to compare the overall observed phenotype distribution and that expected according to Hardy-Weinberg was not possible, see Table 69.

Although little variation was found no differences appear to exist between the six samples tested for this locus. However all these samples were from the North and Irish Seas; the Icelandic samples were not tested for this enzyme.

(g) ADA

Seven samples were tested for this enzyme and details of numbers of different phenotypes found in each sample are given in Table 71; as before the numbers of different types expected according to Hardy-Weinberg are given in parentheses. X^2 tests were possible for all the samples and in five of the seven agreements between observed and expected distributions suggested genetic equilibrium. In two of the samples, number 4, from the Gabbard, and number 9, from the Irish Sea, both collected in 1977, significant deviation from genetic equilibrium was found. In both cases this was due to excesses of phenotype 4 and phenotype 6 individuals and a deficiency of 4-6 heterozygotes.

Details of allele frequencies for all the samples are given in Table 72. Although allele 4 was always the most common some differences were present in the values, both for this allele and allele 2. A contingency test to examine allele distribution throughout the samples indicated heterogeneity (X^2 = 24.67, D.F. = 12, $0.50 > P > 0.02$), see Table 73. This was due to a deficiency of allele 4 and an excess of rarer alleles in sample 10, collected from the Scarborough Ground in 1977. When the test was repeated excluding this sample homogeneity was shown between the

Table 71 ADA:- The numbers of types are shown for each of the seven samples. The expected numbers in parentheses assume perfect genetic balance at each position sampled.

Phenotypes	Year and Locality						
	1977						
	Gabbard (4)	Moray Firth (8)	Maughold Hd. (9)	Scarboro' Gd. (10)	Lowestoft (13)	Cleethorpes (14)	Filey (15)
2 - 2	1 (2.18)	1 (.45)	1 (1.50)	1 (1.56)	1 (1.06)	4 (2.54)	- (.59)
4 - 4	128 (125.34)	33 (32.49)	26 (22.68)	9 (8.90)	98 (101.44)	65 (62.89)	32 (31.74)
6 - 6	21 (11.36)	3 (3.31)	4 (1.50)	1 (.75)	3 (4.39)	6 (4.23)	3 (1.18)
1 - 2	-	-	-	-	-	1 (.27)	-
1 - 4	-	-	-	-	1 (.74)	1 (1.32)	-
2 - 3	1 (.71)	-	-	-	1 (.30)	-	- (.10)
2 - 4	38 (33.03)	7 (7.60)	11 (11.67)	7 (7.46)	21 (20.74)	23 (25.29)	10 (8.66)
2 - 5	- (.98)	- (.06)	1 (.33)	- (.24)	- (.45)	- (.67)	1 (.39)
2 - 6	8 (9.95)	2 (2.43)	4 (3.00)	2 (2.17)	4 (4.32)	6 (6.55)	1 (1.67)
3 - 4	7 (5.39)	-	-	-	3 (2.96)	-	1 (.72)
4 - 5	10 (7.40)	- (.70)	1 (1.29)	1 (.57)	6 (4.44)	5 (3.33)	3 (2.89)
4 - 6	61 (75.48)	21 (20.74)	6 (11.67)	5 (5.17)	47 (42.22)	30 (32.60)	10 (12.26)
5 - 6	1 (2.23)	1 (.22)	- (.33)	- (.17)	- (.92)	- (.86)	- (.56)
6 - 7	-	-	-	-	-	1 (.17)	-
Rest	(1.85)	(.00)	(.03)	(.01)	(.94)	(1.28)	(.24)
Total	276	68	54	27	185	142	61
No. of classes after pooling	10	6	7	6	9	9	7
D.F.	5	2	3	2	3	3	2
χ^2	16.68	.41	7.95	.21	2.27	3.51	3.77
Probability	0.01>0.001	0.90>0.80	0.05>0.02	0.95>0.90	0.70>0.50	0.50>0.30	0.20>0.10

Table 72 ADA:- Frequencies of alleles found in seven samples from different localities.

Year and Locality	Allele							No. of fish tested
	1	2	3	4	5	6	7	
1977								
Gabbard (4)	-	.0888	.0145	.6739	.0199	.2029	-	276
Moray Firth (8)	-	.0809	-	.6912	.0074	.2206	-	68
Maughold Head (9)	-	.1667	-	.6481	.0185	.1667	-	54
Scarborough Ground (10)	-	.2407	-	.5741	.0185	.1667	-	27
1976								
Lowestoft (13)	.0027	.0757	.0108	.7405	.0162	.1541	-	185
Cleethorpes (14)	.0070	.1338	-	.6655	.0176	.1725	.0035	142
Filey (15)	-	.0984	.0082	.7213	.0328	.1393	-	61

Table 73 A contingency test to examine allele distribution at the ADA locus in the seven samples tested.

Sample	Allele 4			Allele 6			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
4	372	379.54	.1498	112	99.13	1.6970	68	73.33	.3874	552	2.23	0.50> 0.30
8	94	93.51	.0026	30	24.42	1.2750	12	18.07	2.0390	136	3.32	0.20> 0.10
9	70	74.26	.2444	18	19.39	.0996	20	14.35	2.2246	108	2.57	0.30> 0.20
10	31	37.13	1.0120	9	9.70	.0505	14	7.17	6.5061	54	7.57	0.05> 0.02
13	274	254.40	1.5101	57	66.45	1.3439	39	49.15	2.6961	370	5.55	0.10> 0.05
14	189	195.27	.2013	49	51.00	.0784	46	37.73	1.8127	284	2.09	0.50> 0.30
15	88	83.88	.2024	17	21.91	1.1003	17	16.21	.0385	122	1.34	0.70> 0.50
Totals	1118			292			216			1626		

Overall X^2 = 24.67
D.F. = 12
0.02 > Probability > 0.01

remaining samples (X^2 = 16.47, D.F. = 10, 0.10 > P > 0.05).

A similar test of phenotype distribution throughout the samples also showed heterogeneity (X^2 = 22.50, D.F. = 12, 0.05 > P > 0.02), see Table 74; a result of excesses of phenotypes 4 and 4-6 and a deficiency of other types in sample 13, '0' group fish collected from Lowestoft beach in 1976. A repeat of the test without this sample indicated homogeneity (X^2 = 14.18, D.F. = 10, 0.20 > P > 0.10).

These two anomalous samples, numbers 10 and 13, had the extreme high and low values for both allele 2 and allele 4, see Table 72. Once again as all the samples tested for this enzyme were from the North and Irish Seas the occurrence of heterogeneity is not easily explained by geographical isolation. The Icelandic samples were not tested for this enzyme.

Due to the heterogeneity between samples a X^2 test between the overall observed phenotype distribution and that expected according to the Hardy-Weinberg Principle was not carried out.

Table 74 A contingency test to examine phenotype distribution at the ADA locus in the seven samples tested.

Sample	Phenotype 4			Phenotype 4-6			Others			Totals	X^2	Probability
	Obs	Exp	D^2/E	Obs	Exp	D^2/E	Obs	Exp	D^2/E			
4	128	132.74	.1693	61	61.11	.0002	87	82.15	.2863	276	.46	.80> 0.70
8	33	32.70	.0028	21	15.06	2.3429	14	20.24	1.9238	68	4.27	0.20> 0.10
9	26	25.97	.0000	6	11.96	2.9700	22	16.07	2.1882	54	5.16	0.10> 0.05
10	9	12.99	1.2256	5	5.98	.1606	13	8.04	3.0599	27	4.45	0.20> 0.10
13	98	88.97	.9165	47	40.96	1.1827	40	55.07	4.1239	185	6.22	0.05> 0.02
14	65	68.29	.1585	30	31.44	.0660	47	42.27	.5293	142	.75	0.70> 0.50
15	32	29.34	.2412	10	13.51	.9119	19	18.16	.0389	61	1.19	0.70> 0.50
Totals	391			180			242			813		

Overall X^2 = 22.50
D.F. = 12
0.05> Probability > 0.02

DISCUSSION

Fifteen samples of juvenile plaice were electrophoretically analysed. These samples were either larvae or small metamorphosed fish reared in the laboratory from eggs collected on different spawning grounds in the North and Irish Seas and off Iceland, or larger '0' group fish collected by push net from shallow water on three beaches on the East Coast of England. Up to eight loci were studied in each sample. The breeding experiments already described in this thesis had confirmed the genetic control of polymorphism at six of these loci whilst at the remaining two, 6PGD and IDH, the phenotypes of the offspring of crosses made between assumed homozygotes complied with interpretations of simple genetic control.

For each of the enzyme systems the allele and the phenotype distributions of all the samples were compared by contingency tests. Unfortunately the sensitivity of these tests was restricted by the need to pool many classes to obtain statistical validity. Nevertheless significant differences between samples for either one or the other, or both of these distributions were found in all the enzymes except IDH. Only six samples were tested for this enzyme and the level of polymorphism was very low.

There were five loci for which data were available from the North Sea, the Irish Sea and from Iceland, namely G3PDH, PGM, GPI-B, GPI-A and 6PGD. At the first three of these significant differences were found between the Icelandic and the North and Irish Sea samples which is perhaps not surprising considering the geographical remoteness of Iceland from the Continental shelf. However significant differences were also found between the various GPI-B samples from around the United Kingdom.

Heterogeneity was also present at the GPI-A and 6PGD loci but this was due to significant deviations from expected allele and phenotype distributions in one of the North Sea samples, number 1 from the Gabbard.

At the remaining two loci, MDH-A and ADA, no Icelandic samples were included, only samples from the North and Irish Seas. At the MDH-A locus homogeneity in the phenotype but not in the allele distributions was found whilst at the ADA locus heterogeneity was present in both distributions.

Although there was heterogeneity between the various North and Irish Seas samples at all the loci except G3PDH, PGM and IDH, there was no consistent source : different samples were anomalous at different loci. Apart from the significant differences from expected found at the MDH-A locus in sample 9 from the Irish Sea all the significant differences were found in samples from sites represented by other samples, collected in different years, where observed allele and phenotype distributions did agree with those expected. Therefore despite these anomalies the evidence available from the MDH-A, PGM, GPI-A, GPI-B, G3PDH, 6PGD and ADA loci gave no indication of consistent genetic differentiation between the various plaice spawning grounds on the Continental shelf around the British Isles.

As a result of plankton surveys carried out over many years by the Lowestoft laboratory it is known that the samples collected for this study represent the main North Sea spawning grounds. Although the centres of these spawnings are known to be separated by time and position, in some instances the limits do overlap (Harding, Nichols and Tungate, 1978; Simpson, 1959).

Consequently the populations may not be finite enough for any genetical isolation to take place. This is almost certainly the case for the samples representing spawning grounds from the southern half of the North Sea. However the Flamborough Off and Moray Firth spawnings are some distance from these.

Tagging experiments carried out over many years have been reviewed by Harden Jones (1968) and he suggests that in the North Sea juvenile fish return to spawn on their ground of origin and that, although the adult fish disperse over a wide area after spawning, the majority, but not all, return to the same spawning ground each year. Hence some interchange of adults between grounds may take place. Although the samples from the North Sea and from the Irish Sea were obviously geographically remote, spawning grounds occur throughout the Channel and the southern Irish Sea and migration of adults may take place between these grounds. In the absence of differential selection the rate of migration from one population to another need only be slight to counteract any genetic drift and to maintain genetic uniformity (Wright, 1940). Migration rates of 0.001 per cent of the population per generation have been quoted in this context for populations of moderate size (Lewontin, 1974). The large size of plaice populations reduces the likelihood of genetic drift and low selection pressures may also be negated by migration. Therefore although some centres of spawning may be isolated by time and position there may be sufficient interchange of adults to maintain genetic uniformity. The genetic homogeneity shown here between the samples from different areas of the North and Irish Seas supports this. In contrast the differences observed between these samples

and those from Iceland reflect the isolation of the plaice stocks around Iceland from those of the Continental shelf.

For each enzyme in each sample the observed phenotype distributions were compared to those expected according to Hardy-Weinberg . However due to either low levels of polymorphism or small numbers of the different phenotypes it was not always possible to carry out X^2 tests between these distributions. Nevertheless in 29 of the 35 tests that could be carried out compliance was found with the possibility that the samples represented populations in genetic equilibrium, maintained by random segregation of alleles within a breeding unit. In the remaining six analyses significant deviations from the expected distributions were due to excesses of homozygotes in the samples. Three of these were found in sample 4, from the Gabbard, at the G3PDH, MDH-A and ADA loci, two were found in sample 10, from the Scarborough Ground, at the MDH-A and PGM loci and one, at the ADA locus in sample 9 from Maughold Head; all three samples were collected in 1977. However there was a trend throughout all the samples and at all the loci for the numbers of homozygotes observed to be greater than expected according to the Hardy-Weinberg distribution although in the majority of cases it was either not possible to test these differences statistically or, when tested, these differences were not significant.

Similarly when all the samples, excluding the two Icelandic ones, were pooled and the resulting phenotype distributions compared with Hardy-Weinberg expectations, excesses of homozygotes were observed at all the loci except IDH, although only at the PGM locus was the excess significant, see Table 75. A similar

Table 75 The total numbers of homozygotes and heterozygotes observed in the North and Irish Seas samples and compared to those expected according to the Hardy-Weinberg Principle.

Locus	Homozygotes		Heterozygotes		X^2	Probability
	Obs	Exp	Obs	Exp		
G3PDH	712	694.74	182	199.26	1.92	0.20>0.10
MDH-A	1396	1367.94	331	359.07	2.77	0.10>0 .05
PGM	1138	1081.36	980	1036.64	6.06	0.02>0.01
GPI-A	1539	1530.53	188	196.47	.41	0.70>0.50
GPI-B	1498	1491.94	229	235.06	.18	0.70>0.50
6PGD	1643	1635.05	84	91.95	.73	0.50>0.30
IDM	814	814.18	20	19.82	.00	P > 0.99
ADA	442	419.38	371	393.62	2.52	0.20>0.10

trend was obtained when any sample which had deviated significantly from homogeneity in the contingency tests of allele and phenotype distributions throughout the samples was excluded from these overall totals, see Table 76, although here a significant excess of homozygotes was also found at the ADA locus. An examination of the observed deviations from expected in the individual phenotype classes shows that, for homozygotes, excesses were consistently observed but that for heterozygotes there was no such simple trend observed for deficiencies, see Table 77. The exclusion of any anomalies, as in Table 76, did not affect this pattern. Applying Levene's Exact test (Levene, 1949) to the totals instead of the Hardy-Weinberg Principle did not alter the expected number of homozygotes in any classes at any locus by more than 0.25 and never affected the significance of the distributions.

Significant excesses of homozygotes have been interpreted as evidence of a Wahlund effect, a mixing of more than one genetically distinct population. However this would not appear to be the case with these data. Discounting the Icelandic samples again, the allele frequencies throughout the remaining samples were similar at all loci. Yet, discussing this effect Li (1969) pointed out that, when a multiallelic locus is being considered in a sample containing a mixture of two populations with different gene frequencies, all classes of homozygotes will appear as excesses but that there will be no such simple trend in the heterozygotes, some classes may appear as excesses whilst others appear as deficiencies. Just this pattern appears in the pooled

Table 76 The total numbers of observed homozygotes and heterozygotes observed in the North and Irish Seas samples, excluding any anomalous samples, are compared to those expected according to the Hardy-Weinberg Principle.

Locus	Homozygotes		Heterozygotes		X^2	Probability
	Obs	Exp	Obs	Exp		
G3PDH	712	694.74	182	199.26	1.92	0.20 > 0.10
MDH-A	1227	1211.75	281	296.24	.98	0.50 > 0.30
PGM	1138	1081.36	980	1036.64	6.06	0.02 > 0.01
GPI-A	1328	1319.43	146	155.57	.66	0.50 > 0.30
GPI-B	998	993.62	152	156.38	.14	0.80 > 0.70
6PGD	1313	1309.13	60	63.87	.25	0.70 > 0.50
IDH	814	814.18	20	19.82	.00	P > 0.99
ADA	328	303.28	273	297.72	4.07	0.05 > 0.02

Table 77 The distribution of differences between observed and expected numbers of phenotypes in all classes of homozygotes and heterozygotes at all the loci in all the North and Irish Seas samples.

Locus	Homozygotes		Heterozygotes	
	Obs > Exp	Exp > Obs	Obs > Exp	Exp > Obs
G3PDH	3	0	2	3
MDH-A	2	0	1	2
PGM	2	0	3	4
GPI-A	2	0	3	3
GPI-B	2	1	3	4
6PGD	1	0	0	3
IDH	0	1	3	0
ADA	3	0	4	6
Totals	15	2	19	25

data of this study and, of course, some Wahlund effect will certainly arise from combining samples with even minor insignificant gene frequency differences. However the significant excesses of homozygotes in three of the samples from different spawning grounds and the general excess in the other samples suggests that an alternative explanation of the results observed here may be necessary. Of course it is theoretically possible that there is also some genetic differentiation within the various sampling sites. However the Hardy-Weinberg test is not very sensitive to deviations from genetic equilibrium, particularly in small samples (Wallace, 1968).

The presence of low frequencies of null alleles could cause an apparent excess of homozygotes as heterozygous individuals containing a null allele could be mistakenly typed as being homozygous for the other allele present. Computing expected phenotype distributions from the resulting inaccurate gene frequencies could lead to discrepancies between the observed and expected values which would be indicated as an apparent excess of homozygotes in the observed distribution. However the presence of null alleles should be electrophoretically detectable by either the lack of enzyme activity in individuals homozygous for a null allele or possibly by the differential staining between individuals homozygous for another, positively staining allele and those heterozygous for this allele and a null. Unfortunately however the frequencies of such alleles need only be very low to create apparent excesses of homozygotes. Even at the PGM locus where the excess of homozygotes was significant, only

57 more homozygotes were observed than expected out of a total of 2018 fish. Assuming these to be misidentifications of individuals heterozygous for a null allele the frequency of such a null would only have been .0135 ; hence out of a total of 2018 fish only 0.37 homozygous null individuals would be expected. However the results observed, either in the breeding experiments or in the population analysis never suggested the presence of such alleles at any of the loci. Also apart from the early G3PDH experiments and ADA experiments no difficulties were experienced in interpreting electropherograms : any such continuous difficulty could have caused the presence of null alleles to be overlooked.

These excesses of homozygotes found in plaice are not unique in marine species. Crisp (1978) discussed the reasons for their common occurrence in littoral marine invertebrates and suggested that besides misinterpretation of electrophoretic results, null alleles and the Wahlund effect, selection for different homozygotes for different environmental niches may be involved. In fish Jamieson (1975) reported homozygote excesses at the transferrin locus in populations of the cod, Gadus morhua but eliminated these excesses in a reanalysis of the samples using a more sophisticated electrophoretic technique which resolved more alleles (Jamieson 1978). In some instances individuals originally typed as homozygotes became heterozygotes. Similarly excesses of homozygotes were reported by Christiansen et. al. (1974) at an esterase locus in populations of the viviparous blenny, Zoarces viviparus, and Christiansen (1977) after discounting null alleles and the Wahlund effect attributed the cause to juvenile zygotic selection. In a study of 12 polymorphic loci in the killifish, Fundulus heteroclitus, Mitton and Koehn (1975) reported consistent heterozygote deficiency at

two esterase loci, the glucose-6-phosphate dehydrogenase locus and a phosphoglucomutase locus but over all 12 loci there was greater viability of more highly heterozygous individuals. They suggested that selection at other closely linked loci may have influenced survival of different genotypes at the four anomalous loci. Rodino and Comparini (1978) attributed a similar deviation from Hardy-Weinberg expectations at a sorbitol dehydrogenase locus in Mediterranean populations of the European eel, Anguilla anguilla, to some form of election.

It may be that in plaice some form of selection is occurring during the early life cycle and that this is observed as excesses of homozygotes and deficiencies of particular heterozygotes. As no differences were observed between the youngest samples tested, reared in the laboratory, and the '0' group fish any selection must take place during the very early stagesof larval development and does not seem to be affected by hatchery rearing.

A comparison between the observed and expected levels of heterozygosity found in this project and those presented by Ward and Beardmore (1977) for the same loci in a Bristol Channel populations and a North East Irish Sea population showed similarity between both sets of data, with the exception of the GPI-B locus, see Table 78. Ward and Beardmores' data were obtained from '0' group fish collected over several months from beaches on the South Wales coast and from trawl caught fish at sea. The ages of these fish were greater than those of the majority of samples tested in this project and this may have caused the difference at the GPI-B locus although differences in electro phoretic techniques and interpretation of electropherograms may have contributed. Generally the allele frequencies of the North and Irish Seas samples were similar in both sets of data at all eight loci discussed here.

Table 78 A comparison between the heterozygosity levels at eight loci found in two independent plaice studies.

Locus	Ward and Beardmore (1977) Bristol Channel			Ward and Beardmore (1977) N.E. Irish Sea			This study		
	Obs	± S.E.	Exp	Obs	± S.E.	Exp	Obs	± S.E.	Exp
G3PDH	.234	± .009	.232	.238	± .036	.241	.196	± .009	.215
MDH-A	.216	± .009	.219	.208	± .034	.188	.192	± .007	.208
PGM	.483	± .011	.499	.507	± .042	.522	.472	± .007	.501
GPI-A	.110	± .007	.109	.147	± .030	.138	.105	± .005	.108
GPI-B	.058	± .005	.057	.069	± .021	.068	.144	± .005	.145
6PGD	.047	± .003	.049	.049	± .013	.047	.048	± .004	.052
IDH	.026	± .004	.026	.042	± .013	.041	.024	± .004	.024
ADA	.418	± .007	.424	.410	± .029	.434	.456	± .012	.456

GENERAL DISCUSSION

The family studies described in this thesis supported the hypothesis that, in plaice, polymorphisms at six enzyme loci are controlled by codominant alleles segregating in Mendelian ratios. At three of these loci, G3PDH, MDH-A and ADA, the results were all consistent, although admittedly at the last locus only limited data were available.

At the PGM locus some significant deviations from expected were observed. However the segregation ratios in the majority of crosses did comply with Mendelian ratios and it may reasonably be concluded that the control of polymorphism at this locus is similar to that of the previously mentioned three loci.

The results of crosses at the GPI-A locus, whilst generally supporting Mendelian inheritance, contained significant deviations from expected in several 2-2 x 1-2 crosses. In these crosses the 1-2 heterozygous offspring appeared to be less fitted for survival than the homozygous 2-2 offspring. The same suggestion was made by Purdom *et. al.* (1976) but their data were very limited and hence their results inconclusive. The experiments described in this thesis not only demonstrated differential survival of the two phenotypes but also showed that any selection must take place very early on in the embryonic phase of the life cycle, before the larvae are ten days old.

In the laboratory experiments the mortality was considerable and the selective effect of the hatchery conditions unknown. However the results of plankton surveys of the southern North Sea have shown that in plaice the mortality rate is most severe, about 93%, during the egg and early larval stages (Harding *et. al.* 1978). In the sea predation may be a significant factor but it may be that

selection pressures are also greatest during these stages.

The majority of crosses made at the GPI-B locus indicated simple genetical control of polymorphism although anomalies were found. The results suggested that some differential fitness between the morphs may have been present in some crosses but the data were too limited to draw any substantial conclusions. At the two other loci examined 6PGD and IDH, no crosses involving heterozygotes were made but all the homozygous crosses complied with the assumption of simple Mendelian control.

The inheritance of five of these enzymes, G3PDH, MDH-A, PGM, GPI-A and GPI-B, was also studied in induced diploid gynogenomes produced from heterozygous females. Recombination frequencies were found for each enzyme and the range of these values showed that diploidy was caused by retention of the polar body of the second meiotic division. The recombination frequencies observed at the PGM locus were higher than expected and were reasoned to be caused by interference between the locus and the centromere. Although the mean cross-over value of 0.21 obtained from all five loci was very similar to that estimated from the carp data of other workers (Golovinskaia, 1968; Cherfas, 1977; Cherfas and Truveller, 1978; Nagy et. al. 1978) the values between broods at each individual locus were less variable in this study. This may have been due to the use of codominant biochemical markers in larval fish. In the carp studies both physical and biochemical markers in older fish were used. Consequently there were problems with the identification of the offspring into different genotype classes and with mortality during rearing, which limited the amount and accuracy of the data obtained.

Earlier workers, studying colour patterns controlled by sex-linked loci, had reported much lower cross-over frequencies in fish. Winge (1927) found frequencies of up to 0.1 in Lebistes reticulatus whilst Gordon (1937) observed values of only 0.01 in Xiphophones maculatus. On the basis of these early findings Purdom (1969) suggested that, in teleosts, one generation of induced diploid gynogenesis caused by the retention of the second polar body would be equivalent to 14 generations of full sib-mating. However the consistently higher crossover values obtained for plaice reduce this estimation of the effect of induced diploid gynogenesis to between three and four generations of sib-mating. Despite this re-estimation the potential remains for the production of inbred lines by this technique.

In the induced triploid offspring the presence of genotypes with three alleles confirmed their triploid state. The recombination frequencies observed were similar to those found in the induced diploid gynogenomes, therefore supporting the conclusion reached for the contribution gynogenesis may make in fish cultivation.

Significant deviations from expected 1 : 1 segregation ratios were observed in the homozygote classes in some of the gynogenetic experiments : but there were no obvious consistent trends for any particular genotype. Indeed, although anomalies were observed throughout the conventional, gynogenetic diploid and induced triploid broods the only consistent deviations were those at the GPI-A locus in the conventional crosses.

A study of the occurrence of these same polymorphic enzyme systems in naturally occurring populations was also undertaken. Since the genetics of these polymorphisms was known it was

unnecessary merely to assume their inheritance from comparison with the results from other domestic species, as has generally been the case in previous studies of commercially important fish populations. Eggs were collected from different spawning grounds around the United Kingdom and Iceland, reared to the larval stage in the laboratory and then examined, thus demonstrating the coordination of a hatchery technique with a population analysis.

Until this study, investigations into fish population genetics have been made using only more mature individuals though sampling throughout all stages of the life cycle, including larvae, has already been suggested (Jamieson, 1974; Thompson and Mostert, 1974,). This is because the collection of very young fish larvae at sea and their storage for testing by electrophoresis onshore is both time consuming and difficult. In plaice, in particular, larger metamorphosed larvae are never caught at sea and sampling becomes possible only when the juvenile fish migrate, later in the year, to the shallow water on beaches. By this time fish from different spawning grounds may have become mixed. Moreover in general, irrespective of species, population studies made upon older fish cannot provide precise information on the age and origins of individuals and these factors may be important in assessing the contributions of different year classes and/or breeding populations to a fishery. Such data may be required if biochemical genetics are to be applied in the management of fish stocks. Collecting eggs within a few days of spawning and typing the resulting hatchery reared larvae can provide some of this information.

Although in this study some genetic differences were observed between individual North Sea samples the overall impression was that the plaice stocks around the United Kingdom are genetically similar and distinct from those at Iceland. The suggestion made earlier in this theseis from the adult plaice data, see page 52, that differences between North Sea fishing grounds may occur at the G3PDH locus were not confirmed by the more extensive data available from this population study.

In fact at all the loci examined the allele frequencies of the larval plaice in the population study were similar to those in the adults collected for use in the breeding experiments. No age-data were available for these adults so an analysis by year class was not possible. However in plaice sexual maturity is not reached until at least two years in males and three years in females. The allele frequencies observed in the larvae, the '0' group fish and the adult fish are listed in Table 79.

When the observed phenotype distributions were compared to those expected according to Hardy-Weinberg equilibrium consistent excesses of homozygotes were found in the larval and '0' group samples. These were not present in the adult fish, see Table 80. Similarly the excess of homozygous individuals observed in different phenotype classes at all loci in the larval and '0' group fish was very considerably reduced in these adults, see Table 81. In fact excesses were now observed in the majority of the heterozygous classes.

In their study of a Bristol Channel plaice population Beardmore and Ward (1977) found that the overall heterozygosity level at five loci, G3PDH, MDH-A, PGM, GPI-A and ADA, altered with age. A

Table 79 The overall allele frequencies observed at eight loci in the larval (L), '0' group (0) and adult (A) fish are compared.

Locus		Allele												
		1a	1	2a	2	3	4a	4	5a	5	6a	6	7a	7	8
G3PDH	L	-	.0587	-	.8728	.0654	-	.0029							
	0	-	.0535	.0013	.8838	.0587	.0013	.0013							
	A	.0037	.0572	-	.8985	.0369	-	.0037							
MDH-A	L		.0060		.8819	.1121		-							
	0		.0051		.8869	.1080		-							
	A		.0043		.8693	.1250		.0014							
PGM	L		.0139		.0038	.3609		.6157		.0058					
	0		.0103		.0103	.3393		.6337		.0064					
	A		.0099		.0057	.3963		.5830		.0043					
GPI-A	L	.0067	.0344		.9383	.0202		.0004							
	0	.0064	.0386		.9486	.0026		.0039							
	A	-	.0298		.9545	.0156		-							
GPI-B	L		.0004		.0041	-		.0467	-	.0007		.9208	-	.0269	.0004
	0		-		.0026			.0257	.0013	-		.9537	.0026	.0141	-
	A		-		.0043	-		.0511	.0014	.0014		.9261	-	.0156	-
6PGD	L		.0194		.9723	.0082		-							
	0		.0167		.9743	.0077		.0013							
	A		.0129		.9835	.0037		-							
IDH	L		.0011		.9877	.0112		-							
	0		.0013		.9884	.0078		.0026							
	A		-		.9906	.0094		-							
ADA	L				.1071	.0094		.6671		.0167		.1988		-	
	0				.1005	.0064		.7101		.0193		.1585		.0013	
	A		-		.1267	.0032		.7240		.0097		.1331		.0032	

Table 80 The total numbers of homozygotes and heterozygotes observed in the adult parent fish are compared with those expected according to the Hardy-Weinberg Principle.

Locus	Adult fish				X^2	Probability
	Homozygotes		Heterozygotes			
	Obs	Exp	Obs	Exp		
G3PDH	220	220.06	51	50.94	.00	P> 0.99
MDH-A	276	271.51	76	80.49	.32	0.70>0.50
PGM	186	175.30	166	176.70	1.30	0.30>0.20
GPI-A	320	321.14	32	30.86	.05	0.90>0.80
GPI-B	304	303.08	50	50.92	.02	0.90>0.80
6PGD	263	263.15	9	8.85	.00	P> 0.99
ADA	87	85.93	67	68.07	.03	0.90>0.80

Table 81 The distribution of differences between observed and expected numbers of phenotypes in all classes of homozygotes and heterozygotes found at all the loci in all the adult samples.

Locus	Homozygotes		Heterozygotes	
	Obs > Exp	Exp > Obs	Obs > Exp	Exp > Obs
G3PDH	2	0	4	1
MDH-A	2	0	2	1
PGM	3	0	3	4
GPI-A	0	1	2	0
GPI-B	1	1	4	1
6PGD	0	1	2	0
IDH	0	1	3	0
ADA	2	1	4	2
Totals	10	5	24	9

decrease from the initially observed heterozygosity level occurred until the age of the fish sampled reached six months when this trend was reversed and increases in heterozygosity were observed. These changes were not great enough to cause significant differences in allele frequencies nor significant deviations from Hardy-Weinberg expectations. The data were obtained from samples collected by push net on beaches and Beardmore and Ward suggested that the initial level of heterozygosity was due to differential migration; the more heterozygous individuals, having a more efficient rate of development, arriving on the beaches first and that the decrease in heterozygosity observed in later samples was caused by the arrival of slower developing homozygotes. The reversal of this trend in older fish was interpreted as selection for heterozygosity.

Comparisons between the data available from this thesis and those of Beardmore and Ward (1977) are restricted as not only were samples of different ages examined in the two studies but also not all of the loci used by Beardmore and Ward (1977) were routinely tested here. The hatchery reared larvae were younger than any of Beardmore and Ward's samples whilst the '0' group fish were older than the majority of their material. Even though the samples collected by Beardmore and Ward (1977) all originated from one spawning population, in one year differences were found between fish from three separate beaches. The samples in this thesis were obtained from a wide range of localities over a number of years therefore further analysis and comparisons between the data may not be valid. However the change from the observed excesses of homozygotes in the larval and '0' group samples to closer agreement with Hardy-Weinberg expectations in the adult fish may be due to a

selection effect similar to that observed by Beardmore and Ward.

An examination of the heterozygosity levels at the only three loci to be typed in all the samples in this study, PGM, GPI-A and GPI-B, shows that the overall mean heterozygosity observed in the adults was higher than that of the '0' group fish but that both these groups of fish had a lower level of heterozygosity than the larval samples, see Table 82. The increase between the '0' group fish and the adults fits Beardmore and Ward's theory of increased heterozygosity with age but the high value for the larval samples is difficult to compare because of their hatchery origin.

Thus the aims of the project, as listed in the Introduction, page 9, were achieved with varying degrees of success. The practical problems of fish husbandry and electrophoresis restricted the amount of data in some instances. Although only eight polymorphic enzyme systems were studied their mode of inheritance was demonstrated and these enzymes were used to study induced diploid gynogenesis and induced triploidy and to assess the roles these techniques may have in fish cultivation. Finally, using the same enzymes, a survey was made of spawning concentrations of plaice which demonstrated that although the stocks around the United Kingdom appear to be genetically similar differences exist between these and the plaice stocks at Iceland.

Table 82 The heterozygosities are compared at the three loci tested in all the samples. Standard errors are given.

Locus	Larval fish	'0' group fish	Adult fish
PGM	.4598 ± .0085	.4756 ± .0130	.4716 ± .0188
GPI-A	.1151 ± .0062	.0874 ± .0101	.0909 ± .0153
GPI-B	.1472 ± .0068	.0812 ± .0098	.1412 ± .0131
Overall	.7221	.6442	.6980

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